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# **Effect of different irrigation systems on nitrous oxide emissions from urine applied to pasture soil**

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A thesis  
submitted in partial fulfilment  
of the requirements for the Degree of  
Master of Agricultural Science

at  
Lincoln University  
by  
May Tana Hedges

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Lincoln University  
2017

Abstract of a thesis submitted in partial fulfilment of the  
requirements for the Degree of Master of Agricultural Science.

## **Effect of different irrigation systems on nitrous oxide emissions from urine applied to pasture soil**

by

May Tana Hedges

Nitrous oxide ( $\text{N}_2\text{O}$ ) is one of the important greenhouse gases (GHGs) that contributes to climate change and depletion of the ozone layer. Nitrous oxide is produced by nitrification and denitrification processes in soils. In New Zealand, the agriculture sector produces the largest proportion of the total GHG emissions and the largest source of  $\text{N}_2\text{O}$  emissions is from agricultural soils. New Zealand's commitment to the Paris Agreement 2015 is to reduce all GHG emissions to 30 percent below 2005 levels, by 2030. Increased GHG emissions have an effect on climate change which is a threat to many countries in the world and especially the South Pacific Islands. These islands have already been affected by climate change where coastal agricultural land is submerged due to sea-level rises. As agriculture in New Zealand is the main emitter of GHGs, and because of the impact it has on the South Pacific Islands, more research is required to reduce emissions. Therefore, the objectives of this research are to: 1) quantify the effect of irrigation systems (spray vs roto-rainer vs flood) on  $\text{N}_2\text{O}$  emissions from urine applied to pasture soil and, 2) determine the relationship between  $\text{N}_2\text{O}$  emissions and soil nitrifier and denitrifier population abundance as affected by different irrigation systems.

A field trial was carried out at Lincoln University Research Dairy Farm over a period of 135 days (late summer to early winter) to assess the effect of the different irrigation systems on nitrous oxide emissions from urine applied to pasture soil. The soil used was Templeton sandy loam/Paparua (Udic Haplustepts). The treatments included three irrigation systems: spray, roto-rainer and flood, each with control (water) and/or urine (700 kg N/ha) treatments. Soil

samples were also collected from companion soil blocks and analysed for soil mineral N, nitrifier and denitrifier population abundance.

The results from this research showed that there was no significant difference between the irrigation systems on total N<sub>2</sub>O-N emissions but the different irrigation systems affected the temporal pattern of the N<sub>2</sub>O-N emissions. The irrigation systems did not significantly affect the AOB, AOA or denitrifier abundance, which helps to explain the similar total N<sub>2</sub>O-N emissions between the irrigation treatments. The emission factor (EF<sub>3</sub>) values for these irrigation treatments (2% for spray, 3% for roto-rainer, and 2% for flood) were higher than the New Zealand's specific EF<sub>3</sub> value (1%). This is probably a result of the warm soil temperatures combined with moist soil conditions under irrigation, as supported by the observed positive relationship between soil temperature and N<sub>2</sub>O-N emissions. Relationships between N<sub>2</sub>O-N emissions and soil water content or water-filled pore space (WFPS) were weak because most of the N<sub>2</sub>O-N emissions occurred at moisture contents below field capacity. Importantly, considerable amounts of N<sub>2</sub>O-N can be emitted from urine patches in irrigated pasture despite the soil water content being below field capacity.

**Keywords:** Nitrous oxide, Spray irrigation, Roto-rainer irrigation, Flood irrigation, Nitrification, Denitrification, Ammonia oxidisers, AOB, AOA, Nitrifiers, Denitrifiers, *nirS*, *nirK*, *nosZ*



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# Chapter 1

## Introduction

### 1.1 N<sub>2</sub>O is an important GHG

Nitrous oxide (N<sub>2</sub>O) is one of the important greenhouse gases (GHGs) that contributes to climate change and depletion of the ozone layer. GHGs are very important in regulating the earth's temperature (i.e. the increase in global average surface temperature) and N<sub>2</sub>O plays a big role in this with its high global warming potential (GWP) (Ministry for the Environment, 2009; IPCC, 2014b). The nitrous oxide concentration in the atmosphere has increased from 270 parts per billion (ppb) in the pre-industrial period to around 324 ppb in 2011, which is about 20% higher than the pre-industrial level (IPCC, 2014a). It has a large radiative-forcing potential, with the long term warming potential about 300 times greater than that for carbon dioxide (Cameron *et al.*, 2013). The increase in the concentration of N<sub>2</sub>O is very concerning. Globally, agricultural soils emit the highest percentage of N<sub>2</sub>O (Cameron *et al.*, 2013).

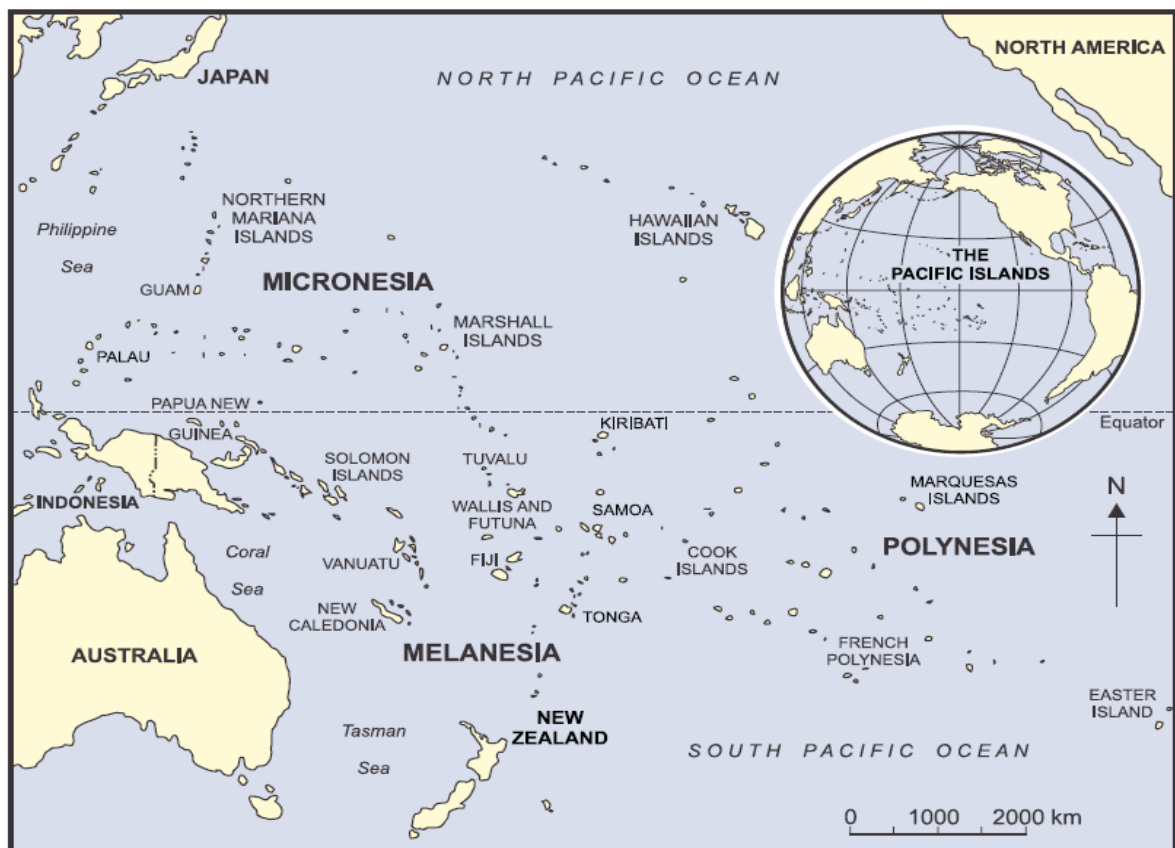
### 1.2 Agriculture's contribution to New Zealand's GHG emissions inventory

Agriculture is the major contributor to the New Zealand economy with 64% of the total value of merchandise exports coming from agricultural products (Ministry for Primary Industries, 2014). As the population of the world increases (from 5.4 billion in the 1990s to 8.5 billion by 2025), an increase in food production (estimation of 60-70% increase) will become necessary to meet the world's demand for food (Bolan *et al.*, 2004). Therefore, the agriculture sector has to expand production to cater for this. There is a great demand for New Zealand agricultural produce in the dairy sector and a favourable milk price has led to an increase in the dairy cattle population and the amount of nitrogen fertiliser applied to agricultural soils. As a result, N<sub>2</sub>O emissions from deposition of urine and dung by the grazing livestock and use of nitrogen fertilizers increased from 2009 to 2012 (Ministry for the Environment, 2015).

The Agricultural sector contributed the largest proportion to New Zealand GHG emissions in 2013 when it contributed 48% of the total GHG emissions. Furthermore, the largest source of N<sub>2</sub>O emissions is from agricultural soils, making a contribution of 94.3% to New Zealand's total N<sub>2</sub>O emissions (Ministry for the Environment, 2015).

### 1.3 GHG emissions (e.g. N<sub>2</sub>O) contribute to climate change which affects the South Pacific Islands and other low lying islands in the world

GHG emissions contribute to climate change which is one of the controversial topics in the world today. Therefore, this can have an impact on farming including (i) increased unpredictability and frequency of extreme weather events such as floods, drought or storms; and (ii) sea-level rises (global mean sea level rise has been observed since the 1950s) submerging coastal agricultural land (IPCC, 2014b). These threats will affect many countries in the world and especially the South Pacific Islands where there is great concern about the impacts. The South Pacific Islands (Fig 1.1) are already affected by climate change. *“Although islanders have done little to contribute to the cause - less than 0.03% of current global greenhouse gas emissions – they are among the first to be affected”* (SPREP, 2014).



**Figure 1.1: A map of the South Pacific Islands (Adapted from Nunn, 2013).**

The Paris Agreement 2015 sets out a goal of limiting the increase in global temperature to well below 2 degrees Celsius because this would reduce risks and impacts of climate change (UNFCCC, 2015). At the Paris Climate Change Conference, New Zealand’s commitment was to reduce its

GHG emissions to 30 percent below 2005 levels by 2030 (M2 Communications, 2015). Research is required to reduce GHG emissions.

#### **1.4 Effect of irrigation and the role of nitrifiers and denitrifiers on N<sub>2</sub>O emissions**

Irrigation is an important means for supplying water for agricultural production as rainfall becomes increasingly less reliable due to climate change (Trost *et al.*, 2013). Improvements in the agricultural activities (e.g. irrigated agriculture) are needed to meet the global food demands of the world's growing population. Soil moisture is a key driver in determining soil N<sub>2</sub>O emissions (Long *et al.*, 2016). Although it has been shown that soil moisture increases after irrigation, in combination with increased mineral N (from deposition of urine or dung on the soil) and can result in significantly higher emissions of N<sub>2</sub>O (Di *et al.*, 2007; Scheer *et al.*, 2008b; Liu *et al.*, 2011), information is limited on the effect of different types of irrigation on N<sub>2</sub>O emissions.

The increase of soil moisture from irrigation may intensify nitrification and denitrification processes and, thus, increase N<sub>2</sub>O emissions (Trost *et al.*, 2013). Ammonia oxidisers, including ammonia oxidising bacteria (AOB) and archaea (AOA) are responsible for carrying out the first step of the nitrification process, the oxidation of ammonia to hydroxylamine (Wrage *et al.*, 2001; Cameron *et al.*, 2013). It has been reported that AOB play a dominant role in high-N status soils, such as in the urine patch areas in grazed pastures (Di *et al.*, 2009, 2010). However, AOA are also present in large numbers in some grazed pasture soils and may also play a role in ammonia oxidation in some soils (Di *et al.*, 2014). Denitrifiers are groups of bacteria which are involved in the stepwise reduction of NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup>, NO, N<sub>2</sub>O and N<sub>2</sub>, producing N<sub>2</sub>O as an intermediate product (Wrage *et al.*, 2001; de Klein *et al.*, 2008; Cameron *et al.*, 2013; Saggar *et al.*, 2013). The reduction of NO<sub>3</sub><sup>-</sup> is catalysed by enzymes encoded by two functional genes (*nirS* and *nirK*) and the reduction of N<sub>2</sub>O was catalysed by enzymes encoded by the *nosZ* gene (Wrage *et al.*, 2001; Jones *et al.*, 2014). Though it has been shown that soil moisture content significantly affects the growth of nitrifiers and denitrifiers in producing higher N<sub>2</sub>O emissions from animal urine treated soils (Di *et al.*, 2014), it is not well understood how different irrigation systems may affect the growth of these key microbial populations, and N<sub>2</sub>O emissions.

## **1.5 Objectives**

The objectives of this research programme were to:

1. Quantify the effect of irrigation systems (spray vs roto-rainer vs flood) on N<sub>2</sub>O emissions from urine applied to pasture soil; and
2. Determine the relationship between N<sub>2</sub>O emissions and soil nitrifier and denitrifier population abundance as affected by different irrigation systems.

## **1.6 Hypothesis**

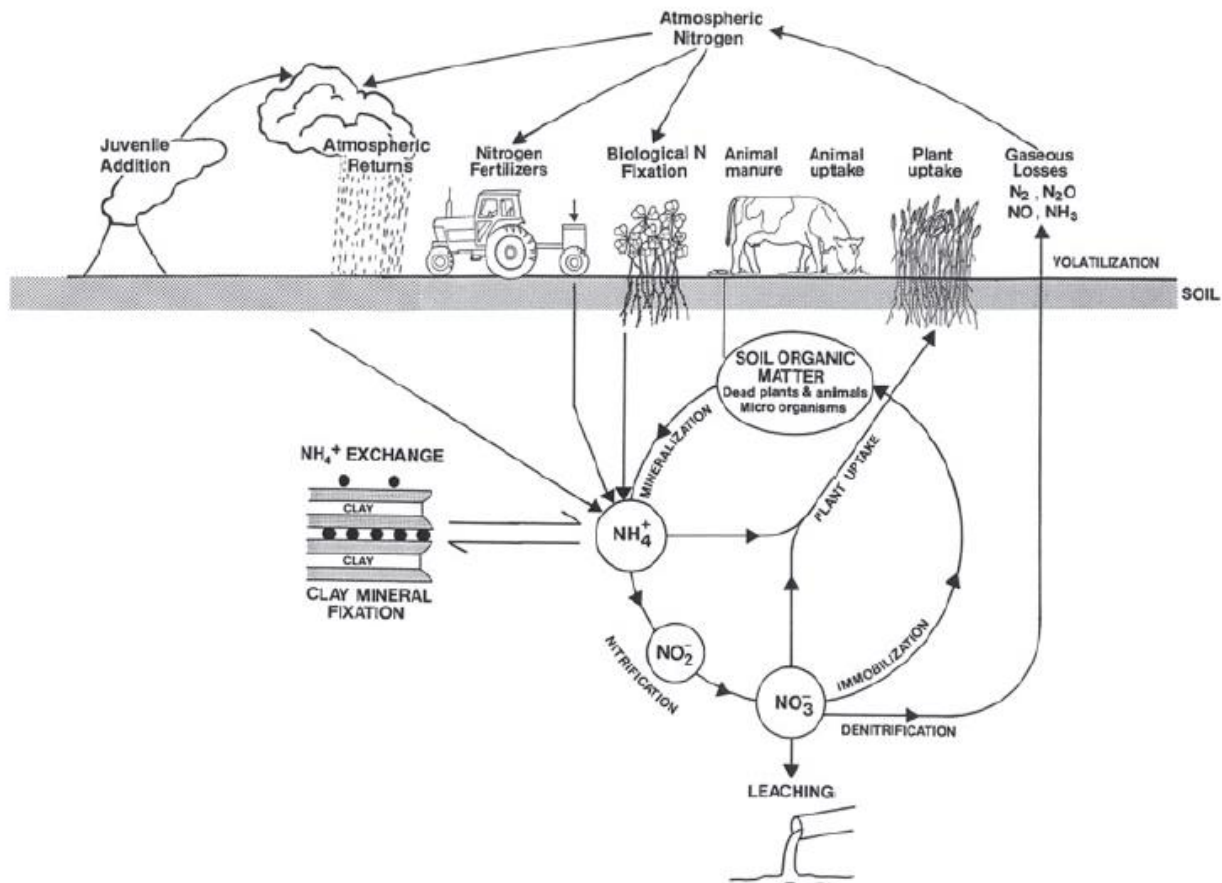
It was hypothesised that:

1. The method of irrigation will affect the N<sub>2</sub>O emissions;
2. The N<sub>2</sub>O emissions will be positively correlated with soil nitrifier and denitrifier abundance.

## Chapter 2

### Literature Review

#### 2.1 The Nitrogen Cycle and Production of Nitrous oxide



**Figure 2.1: The soil/plant nitrogen cycle (Adapted from Cameron, 1992).**

In the nitrogen cycle, nitrogen is cycled from one form to another in the soil/plant/animal system. The amount of nitrogen in the soil is very small compared to the 98% of N held in rocks and minerals. Even though it is small, it provides the bulk of N for plant uptake and eventual animal growth.

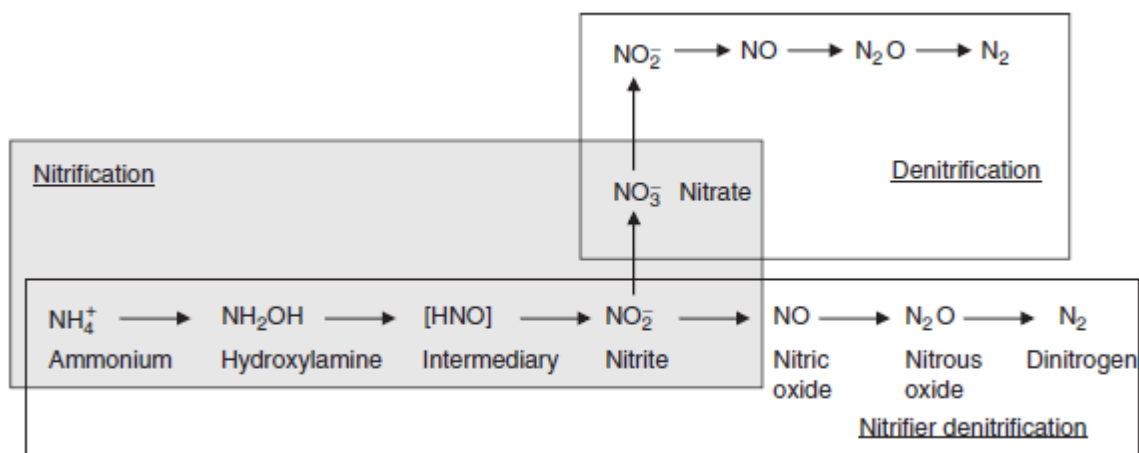
Within the soil/plant/animal system, there are gains, namely atmospheric returns, legume fixation, N-fertilizers and animal manure. Atmospheric returns put N into the soil through wet and dry deposition. Legumes, which fix nitrogen with the help of *Rhizobium* bacteria, increase the level of N in the soil. When N-fertilizer and animal manure (especially animal urine) are applied to the soil, the level of N increases. Within the soil, transformations of N take place which

can make it available for plants or cause it to be lost into the atmosphere or lost by leaching into water.

## 2.1.1 Biological production of Nitrous Oxide (N<sub>2</sub>O)

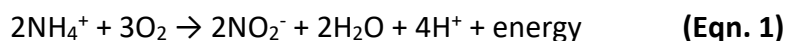
### 2.1.1.1 Nitrification

The Nitrification process produces N<sub>2</sub>O in soils from microbial activity (de Klein *et al.*, 2008; Paustian *et al.*, 2016). It occurs in aerobic conditions (Delwiche, 1981; Cameron *et al.*, 2013). Nitrification is the biological oxidation of ammonium (NH<sub>4</sub><sup>+</sup>) to nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) (Fig 2.2) (Bolan *et al.*, 2004; de Klein *et al.*, 2008; Cameron *et al.*, 2013). N<sub>2</sub>O is produced as an intermediate product of nitrification (de Klein *et al.*, 2008; Paustian *et al.*, 2016).



**Figure 2.2: Schematic representation of N<sub>2</sub>O production from nitrification and denitrification (Adapted from Wrage *et al.*, 2001).**

Nitrification is carried out by a range of microorganisms in the nitrogen cycle. It is carried out by specialised bacteria which oxidise ammonium and is described by the following chemical reactions (Cameron *et al.*, 2013):

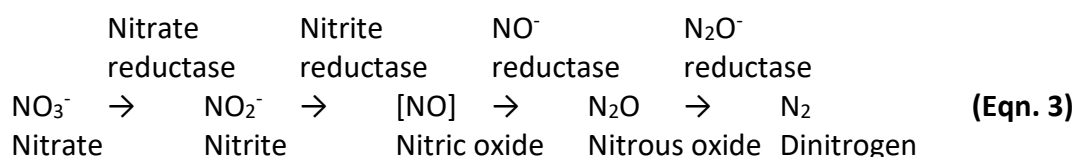


Equation 1 shows the first reaction where NH<sub>4</sub><sup>+</sup> is oxidised to NO<sub>2</sub><sup>-</sup> by ammonia oxidising bacteria (AOB), such as *Nitrosospira* and *Nitrosomonas*. Associated with the bacteria is the ammonia monooxygenase (AMO) enzyme which carries out the oxidation reaction (Ferguson *et al.*, 2007;

Prosser, 2007; Di *et al.*, 2009a, b, 2010a, b). Ammonia oxidising archaea (AOA) are also present in large numbers in soils but their growth is favoured in low N status soils, whereas AOB abundance and activity increases in high N soils (Di *et al.*, 2009b, 2010a, b, 2014; Long *et al.*, 2016). Equation 2 shows the second reaction conducted by *Nitrobacter*, where  $\text{NO}_2^-$  is oxidised to  $\text{NO}_3^-$ . This conversion takes place very rapidly and therefore nitrite rarely accumulates in soil (Cameron *et al.*, 2013). Figure 2.2 also shows the nitrifier denitrification pathway whereby  $\text{NH}_4^+$  is oxidised to  $\text{NO}_2^-$  and then followed by  $\text{NO}_2^-$  being reduced to  $\text{N}_2\text{O}$  and  $\text{N}_2$ .

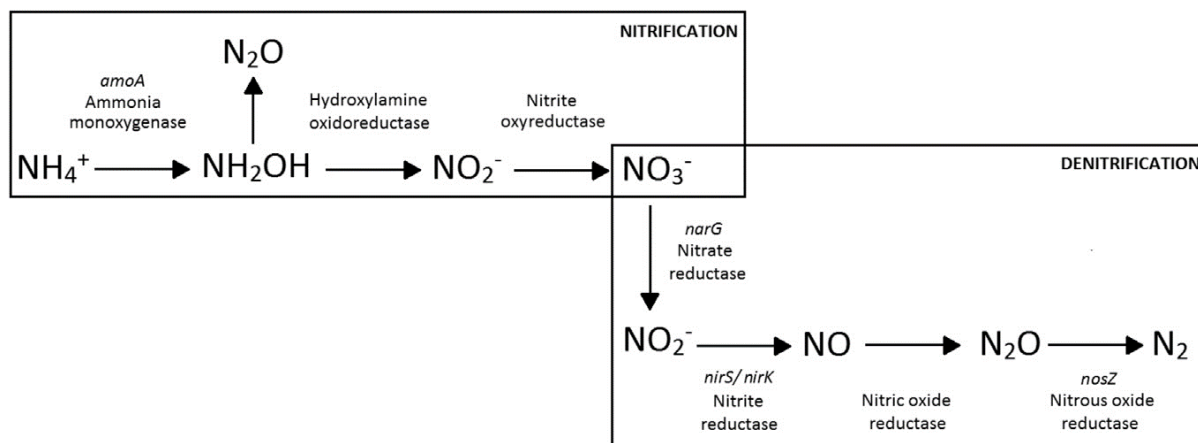
### 2.1.1.2 Denitrification

Denitrification is a stepwise process which is carried out by denitrifying bacteria and each step is catalysed by reductase enzymes namely nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (Wrage *et al.*, 2001; de Klein *et al.*, 2008; Cameron *et al.*, 2013; Saggar *et al.*, 2013) (Equation 3) (Fig 2.3). Biological denitrification and complete reduction of  $\text{NO}_3^-$  to  $\text{N}_2$  has the following key requirements: 1) the presence of microbes harbouring the genetic ability to perform the steps in denitrification, and 2) suitable environmental conditions for expression of the genetic potential (Samad *et al.*, 2016). For example, some bacteria are complete denitrifiers because they contain all the genetic information needed to produce the four enzymes, whereas others are partial denitrifiers because they lack a subset of the enzymes; therefore they are only able to complete part of the reduction (Bakken *et al.*, 2012; Cameron *et al.*, 2013; Regaert *et al.*, 2015).



The genes that are involved in the reduction of  $\text{NO}_2^-$  to  $\text{N}_2\text{O}$  are *nirS* and *nirK* - the first gas producing phase of denitrification, and the final step of denitrification involves the *nosZ* genes which code for the enzyme which reduces  $\text{N}_2\text{O}$  to  $\text{N}_2$  (Treweek *et al.*, 2016a). These genes play an important role during denitrification and have implications for  $\text{N}_2\text{O}$  emissions (Jones *et al.*, 2014) (Fig 2.3).

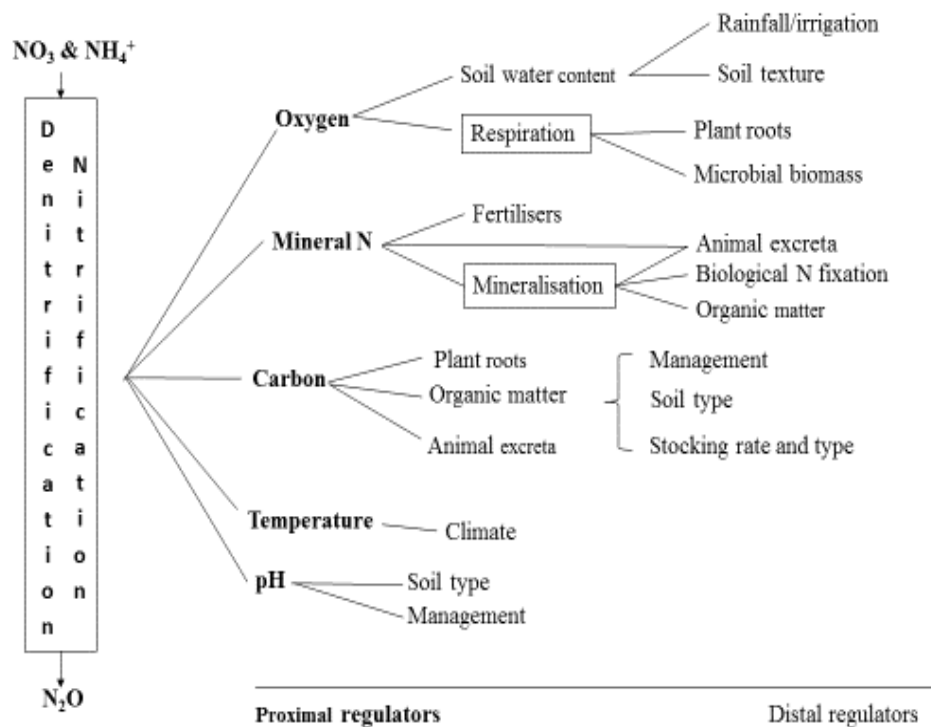




**Figure 2.3: Nitrification and denitrification processes with associated enzymes and functional genes measured in this study (Adapted from Wrage *et al.*, 2001).**

### 2.1.2 Factors affecting $\text{N}_2\text{O}$ emissions and microbial communities in agricultural soils

Nitrification and denitrification are affected by a number of proximal soil factors which in turn are affected by various more distal factors (de Klein *et al.*, 2001) (Fig 2.4). The interaction between these factors can increase or decrease nitrification and denitrification rates. For example, denitrification rates or  $\text{N}_2\text{O}$  emissions are higher following rainfall or irrigation events (de Klein *et al.*, 1999).



**Figure 2.4: Systematic diagram of the factors affecting N<sub>2</sub>O emissions in agricultural soils (Adapted from de Klein *et al.*, 2001).**

### 2.1.2.1 Temperature

Temperature is a major factor that affects N<sub>2</sub>O emissions. Denitrification rates and N<sub>2</sub>O emissions increase with increasing temperatures (Ryden, 1986; Dobbie & Smith, 2001; Saggar *et al.*, 2004). Two examples of the studies carried out found that: denitrification rates increased 10 – fold in a grassland soil when the temperature increased from 10°C to 20°C (de Klein & van Logtestijn, 1996) and in a forest soil, denitrification increased 10 to 20 – fold when the soil temperatures increased from 6°C to 21°C (Nommik & Larsson, 1989). Studies carried out by Dobbie & Smith (2001) and de Klein & van Logtestijn (1996) showed that the effect of temperature on denitrification rates was greater in non-irrigated dry soil compared to irrigated soil. Another study showed that in sandy and loess soils, N<sub>2</sub>O production increased with the soil temperature until 15°C - 20°C and above this temperature range lower emissions were detected (Horváth *et al.*, 2010).

#### **2.1.2.2 Soil drying-rewetting**

For various agricultural systems, it was reported that soil denitrification rates and N<sub>2</sub>O emissions increased following wetting of dry soil by rainfall or irrigation (Kessavalou *et al.*, 1998; Nobre *et al.*, 2001; Kim *et al.*, 2009) including grazed pastures (Luo *et al.*, 1998; Garcia-Montiel *et al.*, 2003; Sagggar *et al.*, 2004a; Kim *et al.*, 2010). The re-wetting of dry soil induces a rapid pulse of N (and C) mineralisation in pasture soils known as the 'Birch effect' (Unger *et al.*, 2010), which increases the amount of N available for nitrification and denitrification to N<sub>2</sub>O (de Klein *et al.*, 2014). The wetting of dry soil enhances the growth and turnover of microorganisms.

#### **2.1.2.3 Soil texture**

Soil texture influences N<sub>2</sub>O emissions (Jamali *et al.*, 2016). Compared with sandy soils, N<sub>2</sub>O emissions are higher in clay soils due to higher denitrification activity because of their slower drainage rate causing anaerobic soil conditions (Luo *et al.*, 2010a; Cameron *et al.*, 2013; Jamali *et al.*, 2016). In contrast, free draining podzols produced higher N<sub>2</sub>O emissions than poor draining gley soils (Rafique *et al.*, 2011). This was a result of enhanced nitrification taking place in the podzols with higher porosity.

#### **2.1.2.4 Soil pH**

Soil pH affects both the nitrification rate and the denitrification rate and as a result this influences the emission of N<sub>2</sub>O and N<sub>2</sub> gas (Parkin *et al.*, 1985; Gødde & Conrad, 2000; Šimek and Cooper, 2002). This also affects the abundance of microbial communities (Mørkved *et al.*, 2007). For example, AOB and AOA prefer to grow in different soil pH environments: the growth of AOB is favoured in neutral to alkaline pH soils and AOA may out-compete AOB in more acidic soils (Robinson *et al.*, 2014). Furthermore, this study found that N<sub>2</sub>O emissions increased when soil pH decreased.

#### **2.1.2.5 Soil mineral nitrogen**

Nitrogen (N) is available in the soil as NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> and this has a big influence on the denitrification process (Cameron *et al.*, 2013; Sagggar *et al.*, 2013). During outdoor grazing, animal excreta (urine or dung) is deposited on the soil and this contributes to large amounts of N in the soil. For example, dairy cows can deposit the equivalent of 1000 kg N per ha in their urine (Haynes & Williams, 1993; Fraser *et al.*, 1994). The increase in the amount of mineral N in the soil induces very large increases in denitrification (de Klein *et al.*, 2001; Di & Cameron, 2003; Di *et al.*, 2007),

which results in the production of  $\text{N}_2\text{O}$ . The proportion of N emitted as  $\text{N}_2\text{O}$ -N from urine-N applied is called the 'emission factor' (IPCC, 2007). The IPCC's default emission factor ( $\text{EF}_3$ ) for  $\text{N}_2\text{O}$  emissions from urine deposited on grazed pasture soil is 2% (de Klein, 2004) and the New Zealand specific default emission factor is 1% (de Klein *et al.*, 2003).

## **2.2 Effect of soil moisture and irrigation on nitrous oxide emissions**

### **2.2.1 Soil moisture and aeration**

Soil moisture can influence  $\text{N}_2\text{O}$  emissions since it can directly regulate oxygen availability in soil pores, which determines the activity of nitrification and denitrification organisms within the soil profile (Zheng *et al.*, 2000). It has been reported that nitrification and denitrification rates were closely related to the water-filled pore space (WFPS) of a soil (Sangar *et al.*, 2011). Generally, nitrification rates are highest when soil moisture content is below field capacity and nearly stops in saturated soils due to lack of oxygen ( $\text{O}_2$ ), whereas denitrification rates generally increase when soil moisture content increases (Davidson, 1992; Maag and Vinther, 1996). It has been reported that  $\text{N}_2\text{O}$  emitted from a silt loam soil at 70% WFPS was produced during denitrification, while at 35-60% WFPS the main process producing  $\text{N}_2\text{O}$  was nitrification (Bateman and Baggs, 2005). In relation to this, Table 2.1 shows that, above a specific soil water content threshold, denitrification rates increased sharply with increasing soil water content and, below this, denitrification rates were not related to soil water content (Bolan *et al.*, 2004).

**Table 2.1: The Effect of Soil Moisture on Nitrous Oxide Emissions from Pasture Soils (Adapted from Bolan *et al.*, 2004).**

Soil type	Country	WFPS <sup>a</sup> /SWC <sup>b</sup>	Observations (N <sub>2</sub> O emissions)	Reference
Loess	Germany	75-97%	Threshold level 88-90%; above which exponential increase in denitrification rates was observed	Prade and Trolldenier (1988)
Silt loam	Lincoln, US	60-90%	Denitrification increased with increase in WFPS; the increase was gradual in sandy soil	Weir <i>et al.</i> (1993)
Silty Clay loam Sand Sand	Netherlands	60-99%	Threshold levels were 82, 83, 71% for sandy, loamy and peat soil, respectively	De Klein and van Logtestijn (1996)
Loam Peat Loam	Sweden	0.10-0.30 g water per g dry soil <sup>b</sup>	Denitrification increased exponentially above 0.16-0.2 g water per g soil	Klemetsson <i>et al.</i> (1991)
Clay Loam	UK	63% 71% 84%	0.46 kg N <sub>2</sub> O ha <sup>-1</sup> d <sup>-1</sup> 0.92 kg N <sub>2</sub> O ha <sup>-1</sup> d <sup>-1</sup> 3.38 kg N <sub>2</sub> O ha <sup>-1</sup> d <sup>-1</sup>	Abbasi and Adams (2000)
Alluvial soil	New Zealand	63-93%	Threshold level 83%; denitrification rate increased when WFPS was above the threshold level	Ruz-Jerez <i>et al.</i> (1994)
Silty clay Silty loam Sandy loam	USA, India	60% 90%	Denitrification losses: 0.02-0.18 mg N per kg soil 14-18.6 mg N per kg soil	Aulakh <i>et al.</i> (1991)
Stagno-Dystic Gleysol	UK	70-90%	50-fold increase in denitrification within 70-90% WFPS	Scholefield <i>et al.</i> (1997)
Clay Loam	USA	50-70%	Denitrification increased with increasing WFPS. At highest WFPS, 10-fold higher denitrification in clay loam compared with sandy loam soil	Sextstone <i>et al.</i> (1988)
Sandy Loam				
WFPS = Water-Filled Pore Space		SWC = Soil Water Content		

Table 2.1 shows that different soil types have different threshold values expressed as water-filled porosity (WFP). The critical WFP for many soils is equivalent to field capacity or above (de Klein

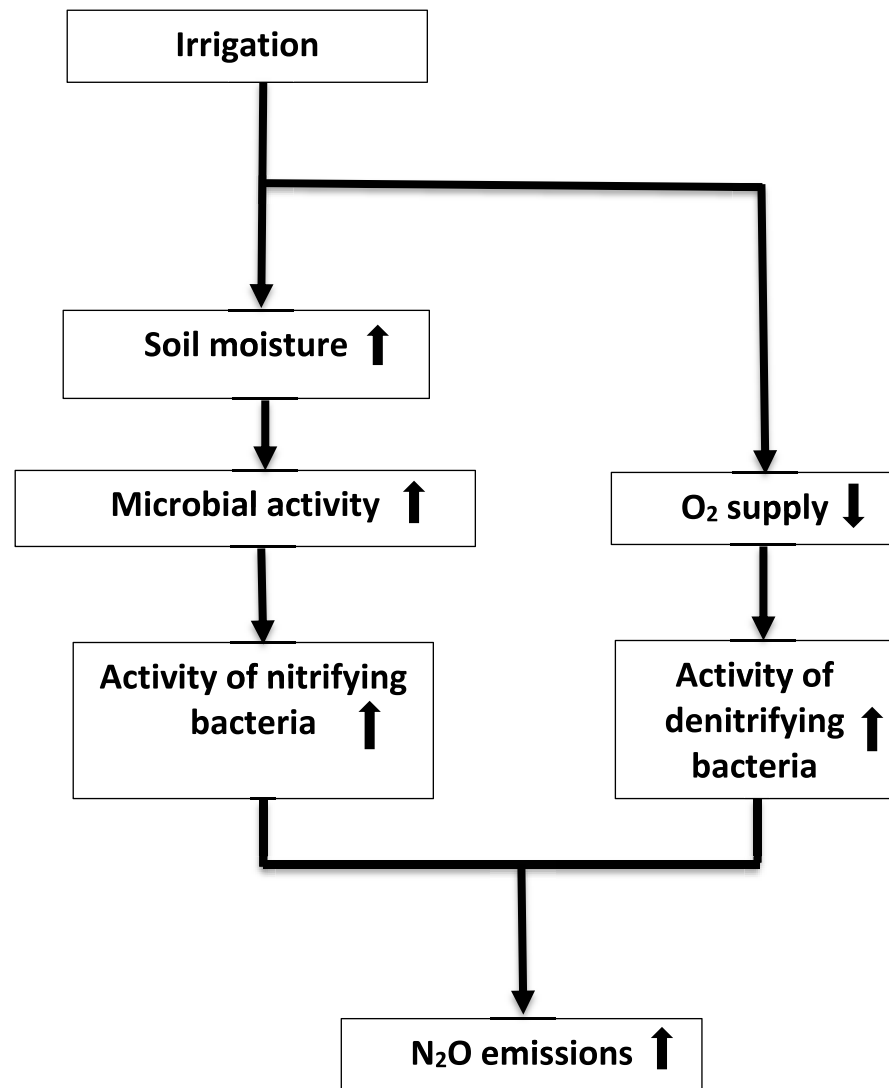
and van Logtestijn, 1996). In general, there was a decrease in water thresholds when soil texture became finer.

When the moisture content of the soil is greater than field capacity there is a significant increase in the potential denitrification rate (de Klein & van Logtestijn, 1996; Muller & Sherlock, 2004). As soils become wetter, they become more anaerobic; therefore this usually increases N<sub>2</sub>O emissions (Dobbie *et al.*, 1999; Dobbie and Smith, 2001). A study carried out by Di *et al.*, (2014) showed that soil moisture content was a major driver for N<sub>2</sub>O emissions from soils treated with animal urine. Furthermore, this study showed that the growth of ammonia oxidiser and denitrifier communities were significantly affected by the soil moisture content and the functional genes increased with increased soil moisture content. As the soil moisture increased, the soil became increasingly anaerobic, leading to higher denitrification rates. Heavy rainfall and irrigation can also cause denitrification (Di & Cameron, 2003).

### **2.2.2 Irrigation**

Irrigation in agriculture plays a vital role in meeting the global food demand of a growing population in the context of climate change (Scheer *et al.*, 2012). Due to climate change, there is an increasing water scarcity which makes efficient irrigation an important means to supply water for crop production and other agricultural activities (Trost *et al.*, 2013). It is estimated that irrigated agriculture will produce nearly two-thirds of future food needs (FAO, 1996). Around the world agricultural land which receives irrigation is estimated to be over 300 million ha (FAO, 2010). In New Zealand the majority of the irrigated land is in the South Island, with Canterbury, at 444, 800 ha which represents approximately 60% of all irrigated area (Statistics New Zealand, 2012). Even though significant amounts of N<sub>2</sub>O are emitted from agriculture, irrigation will always be used. Nitrous oxide emissions from agriculture are therefore projected to grow annually by 50 percent (FAO, 2002).

Irrigation has effects on N<sub>2</sub>O emissions which influence the microbial processes in the soil (Figure 2.5) (Trost *et al.*, 2013). The microbial processes of nitrification and denitrification produce N<sub>2</sub>O (de Klein *et al.*, 2008). As shown in Figure 2.5, when irrigation is applied it increases soil moisture and hence increases the microbial activity of nitrifiers that produce N<sub>2</sub>O. On the other hand, oxygen supply is reduced after application of irrigation which increases the microbial activity of denitrifiers to produce N<sub>2</sub>O. As a rule, N<sub>2</sub>O emissions increase under irrigation when reactive nitrogen compounds are adequately available (Trost *et al.*, 2013).



**Figure 2.5: Basic effects of irrigation on nitrous oxide (N<sub>2</sub>O) emissions (increase, decrease) (Adapted from Trost *et al.*, 2013).**

Appropriate management of irrigation can reduce N<sub>2</sub>O emissions (Maris *et al.*, 2015). Huijing *et al.* (2016) also suggested that controlled irrigation can mitigate the annual integrative global warming potential of N<sub>2</sub>O. Soil moisture can be easily controlled in irrigated systems since it is one of the most important factors to mitigate N<sub>2</sub>O emissions (Scheer *et al.*, 2012).

There have been few studies carried out examining how irrigation affects N<sub>2</sub>O emissions from urine patches (Di and Cameron, 2002). Sanchez-Martin *et al.* (2010) and Kallenbach *et al.* (2010) carried out studies looking at the effects of irrigation systems on nitrous oxide emissions (drip vs furrow irrigation). They found that N<sub>2</sub>O emissions were lower under drip irrigation than under furrow irrigation. The reason is the partial wetting of soil under drip irrigation compared to furrow irrigation which causes the soil to be saturated (Kallenbach *et al.*, 2010). There are,

however, other studies on irrigation applied to cropped systems where irrigation did not have any effect on N<sub>2</sub>O emissions (Simojoki and Jaakkola, 2000; Horváth *et al.*, 2010; Scheer *et al.*, 2013; Maharjan *et al.*, 2014; Owens *et al.*, 2016; Wang *et al.*, 2016). These contrasting studies show that there is a lack of knowledge about the effect of different irrigation systems on nitrous oxide emissions from urine applied to pasture soil.

Therefore the objectives of this thesis are to:

1. Quantify the effect of irrigation systems (spray vs roto-rainer vs flood) on N<sub>2</sub>O emissions from urine applied to pasture soil.
2. Determine the relationship between N<sub>2</sub>O emission and soil nitrifier and denitrifier population abundance as affected by different irrigation systems.



## Chapter 3

### Materials and Methods

#### 3.1 Experimental design and field trial layout

A field experiment was carried out to determine the effect of different irrigation systems on nitrous oxide emissions from urine applied to pasture soil. The experiment consisted of 24 lysimeters which were treated with urine or water (control). Encased in 50 cm diameter by 70 cm deep lysimeters was Templeton sandy loam/Paparua soil (Udic Haplustepts, Soil Survey Staff 1998) which had a standard pasture (rye grass – *Lolium perenne* and white clover – *Trifolium repens*) growing on them (undisturbed soil monoliths with established pasture). These were collected following procedures already established (Cameron *et al.*, 1992; Di & Cameron 2002b). This involved a metal cylinder casing placed on the soil surface, digging around the casing with minimal disturbance to the soil, then gradually pushing the casing down into the soil by small increments. When the casing reached the depth of 70 cm, heated and liquefied petroleum jelly was used to seal the gap between the soil core and the metal casing to stop any edge flow down the lysimeter casing (Cameron *et al.*, 1992). A cutting plate was pushed beneath the lysimeter casing and then cut at the base of the soil monolith. The lysimeter casing was lifted out from the collection site and transported to the Lincoln University's lysimeter facility (Fig 3.1) using a specially designed trailer with air-bag suspension to minimise disturbance to the soil monolith. The lysimeters were then installed in the field lysimeter facility with their surfaces at the same level as the surrounding soil surface, in order to maintain normal growing conditions for the plants (Di & Cameron 2002b).

There were six treatments and four replicates. The treatments were allocated to the lysimeters randomly. The lysimeters were arranged alongside a trench: 12 lysimeters on one side and the other 12 on the opposite side (see Figs 3.1 & 3.2).

In addition to the lysimeters, there were twelve soil blocks (50 cm in diameter and 7.5 cm deep) which were halved to make up 24 sampling blocks. These were collected following procedures already established as described above for the lysimeters (Cameron *et al.*, 1992; Di & Cameron 2002b). Urine was applied to twelve half blocks and water was applied to the other twelve half blocks (control). This is shown in Figures 3.2 and 3.3.

Urea was also applied to the lysimeters and soil blocks (Table 3.1). The amount of urea applied to each lysimeter and soil block was 25 kg N/ha per application. This was applied in split applications over the duration of this experiment to make up the recommended amount of urea (150 kg N/ha) applied annually by farmers.

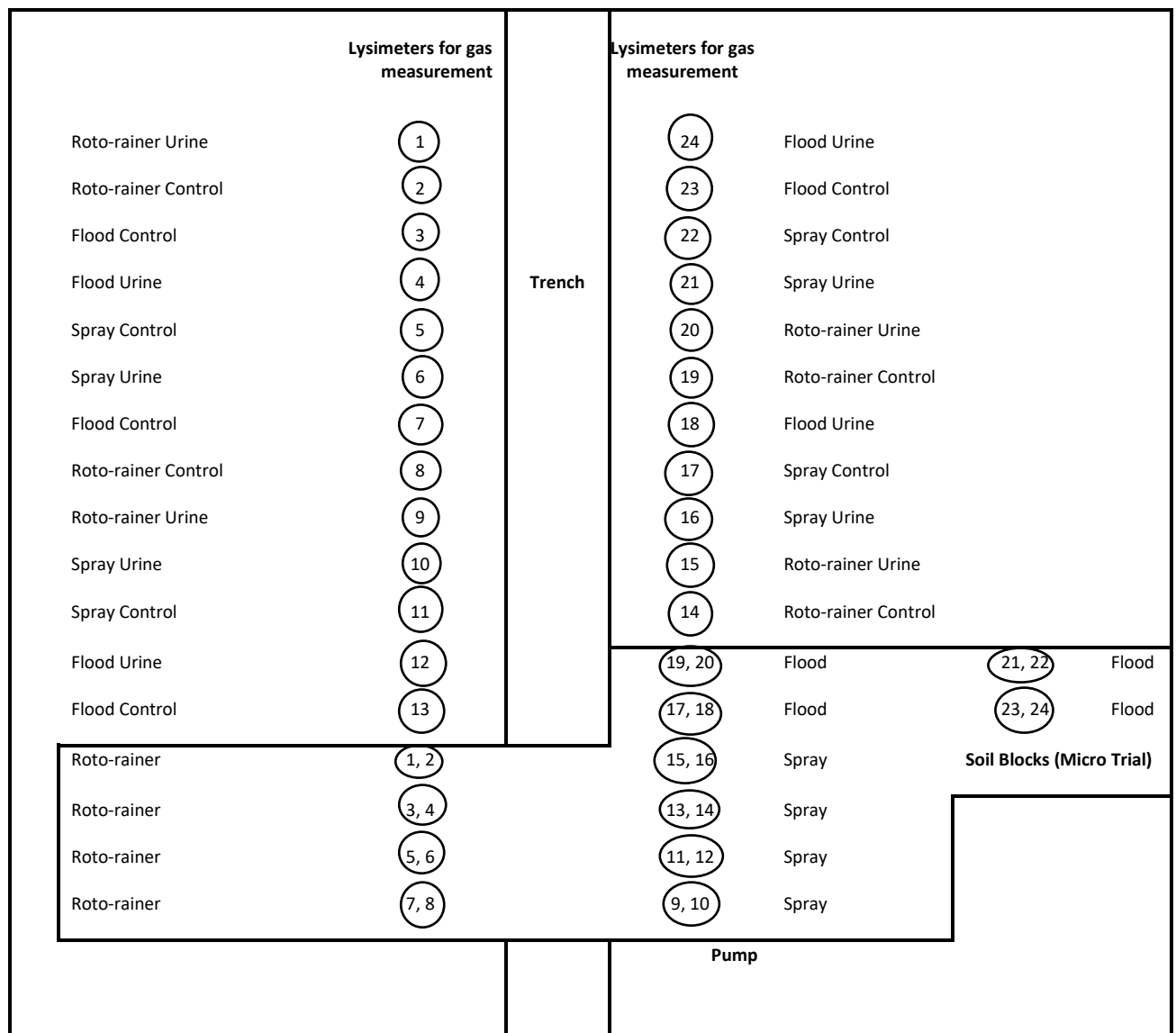
Overhead sprinklers were placed 300 mm above the lysimeters and soil blocks providing irrigation for the pasture. These sprinklers worked in the same way as the irrigation systems used on New Zealand dairy farms. The irrigation systems that were simulated were: 1) spray irrigation - a centre-pivot system which pumps water along arms that travel in a circle across the field (the spread of water is relatively even), 2) roto-rainer irrigation - uses a rotating boom to spread water on the field and, 3) flood irrigation – water is applied by flooding on the soil surface. The schedules for the different irrigation systems are shown in Table 3.1 below.

**Table 3.1: Treatments for the lysimeters and soil blocks**

Treatment #	Irrigation system	Rate (mm)	Frequency	Urine N (kg N/ha)	Urine date	Urea (kg N/ha)	Reps
1	Spray	15	Every 3 days	700	16 Feb	150	4
2	Spray	15	Every 3 days	0	16 Feb	150	4
3	Roto-rainer	45	Every 9 days	700	16 Feb	150	4
4	Roto-rainer	45	Every 9 days	0	16 Feb	150	4
5	Flood	90	Every 18 days	700	16 Feb	150	4
6	Flood	90	Every 18 days	0	16 Feb	150	4



**Figure 3.1: Lincoln University's lysimeter facility.**



**Figure 3.2: Layout of lysimeters for gas measurement and soil blocks in the field.**



**Figure 3.3: Part of the soil blocks with a divider (metal barrier) in the middle of the ring and with the overhead sprinklers.**

### **3.2 Urine collection and application to the lysimeters and soil blocks**

114 litres of fresh urine was collected from cows on the Lincoln University Dairy Farm: that is 60 litres for the lysimeters, 48 litres for the 24 soil blocks and 6 litres spare. This was collected from the Lincoln University Dairy Farm in the afternoon when the cows came in for milking. Urine was collected by filling up a container when the cows urinated. The cows were followed around to see when they wanted to urinate and the container quickly placed close to its rear end. A team of 12 people did the urine collection. The urine was stored in a cool store overnight and applied the next day.

The urine was analysed to determine the N concentration and then standardised to 7 g N/L and applied at the rate of 700 kg N/ha to each of the lysimeters by pouring it into the rings (Fig 3.4).





**Figure 3.4: Pouring urine into the ring on the lysimeter.**

In addition, the standardised urine (7 g N/L) was also applied at the rate of 700 kg N/ha on to each half of the soil blocks. This was poured carefully into one half of the ring as shown in Figure 3.5.



**Figure 3.5: Pouring urine carefully into one half of the ring (soil block) (note the metal barrier between the two halves of the soil block).**

For the control, two litres of water was applied to the lysimeters and one litre of water was applied to each half of the soil blocks. This was to keep the liquid addition consistent in all the control treatments.

### **3.3 Nitrous oxide measurement**

N<sub>2</sub>O emissions from the soil were measured at Lincoln University's lysimeter facility (Fig 3.1). This facility consisted of lysimeters in which treatments could be added and replicated in the open field as described in method section 3.1.

Gas sampling was conducted in the field using the standard closed chamber method similar to that described by Hutchinson and Mosier (1981) (Fig 3.6). The gas chamber was made of a steel cylinder which is insulated on the outside with 2.5 cm thick polystyrene foam to prevent heating of the atmosphere in the chamber during sampling. Before samples were collected, gas chambers were lined up beside the lysimeters, the ring troughs on the lysimeter were filled with water, the syringes prepared by fully pushing the plunger in, and the white pressure cap on top of the chamber removed. At time 1, the metal ring of the chamber was placed inside the water trough fitted around the perimeter of the lysimeter to create an airtight seal. When the chamber had settled, the white pressure cap was replaced. The "a" sample vial (12 mL) was fitted to the needle on the gas chamber and the syringe needle inserted into the vial. The syringe was drawn to capture 60 mL of chamber air making sure the air pressure was equalized before it was discarded to the atmosphere. This was carried out once to rinse the vial before the actual sample was collected. After the air was discarded, chamber air was drawn into the syringe again making sure the pressure had equilibrated before the plunger was pushed back to approximately 25 mL. The chamber tap was turned off and the vial was removed from the chamber needle with the syringe still in the vial. The syringe contents were compressed into the vial, the vial was then removed (now had 12 mL of compressed chamber air) and remaining air was discarded from the syringe and the vial placed in holder. At the next 1 minute interval gas sampling was done on the next chamber. Samples were collected in groups of 12 which allowed eight minutes before the next samples were collected. At the 21 minute and 41 minute sampling times, "b" and "c" vial samples were collected respectively. These were collected similarly to the "a" sample with the exception of the "rinse" step whereby 60 mL of chamber air was drawn into the syringe and then discarded into the chamber (this was repeated at least 2 times). After the rinse, 25 mL of chamber air was drawn and ready to be compressed into the vial. After collecting the "c" sample (end of sampling),

the white pressure caps were undone and the chambers were removed from the lysimeters and placed on the side. At the end of sampling all the chambers were collected and stored on site. Gas sampling was carried out in the middle of the day between 12:00 h and 14:00 h, a time when N<sub>2</sub>O emissions were reported to be representative of the average for the day (de Klein *et al.*, 2003).

Gas sampling was started on the 15<sup>th</sup> of February and was carried out twice a week for the first four months and then once a week until the N<sub>2</sub>O-N flux reached background levels. N<sub>2</sub>O concentrations were determined using gas chromatography (SRI 8610 gas chromatograph; SRI Instruments, California, USA) equipped with a <sup>63</sup>Ni electron capture detector (Dai *et al.*, 2013).



**Figure 3.6: Collecting gas samples from the closed gas chambers.**

### **3.4 Soil Sampling**

Soil sampling was conducted in the morning of Day 1 (a day after urine application), Day 7, Day 14, Day 30, Day 60 and Day 90. Soil sample cores were collected from the twenty-four half soil blocks using a soil corer (Fig 3.7). Three soil sample cores were taken randomly at a depth of 0-10 cm for each treatment and placed in a plastic bag. These were taken back to the lab and refrigerated until use. Five plastic bags of soil cores were taken out of the refrigerator at one time and each bag was mixed thoroughly and stored in vials in the freezer for AOA/AOB and denitrifier assays. The other soil cores were used to measure extractable ammonium and nitrate and also soil moisture. See methods 3.5, 3.6 and 3.7 respectively for the above. After each sampling, the



holes were filled with topsoil and the sampling locations marked to help with future sampling (see Fig 3.8).



**Figure 3.7: Soil corer**



**Figure 3.8: White markers in the soil block indicating the last sampling locations.**

### **3.5 Extractable ammonium and nitrate**

Soil subsamples were taken from the soil cores that had been thoroughly mixed on Day 1, Day 7, Day 14, Day 30, Day 60 and Day 90. These soil subsamples were stored in a fridge until extraction. 4 g of soil subsample was weighed into a 50 mL falcon tube and 40 mL of 2M potassium chloride (KCl) was added and capped. The samples were loaded onto the end over end shaker and shaken for 1 hour. They were removed from the shaker and centrifuged for 10 minutes at 2,000 rpm. The samples were then filtered into 30 mL pottles using Whatman 41 filter papers. The collected filtrates were placed in the freezer until they were analysed using a flow injector analyser (FIA).

### **3.6 AOA/AOB assays**

Soil subsamples were taken at Day 1, Day 7, Day 14, Day 30, Day 60 and Day 90. The samples were mixed thoroughly in their respective plastic bags after breaking them up with a rolling pin. The fine soil was scooped with a spoon, making sure there were no big lumps or plant roots, and put into vials. There were two vials (one for extraction and the other one is spare) for each sample. The soil subsamples were stored in the freezer at -80 °C before DNA extraction.

#### **3.6.1 DNA extraction**

A 0.25 g soil subsample was used for DNA Extraction using NucleoSpin® Soil Kit (Macherey-Nagel, Düren, Germany) according to manufacturer's instructions. The 0.25 g soil subsample was transferred to a NucleoSpin bead tube and 700 µL buffer SL2 and 150 µL enhancer SX were added to adjust conditions for cell lysis. Samples were processed in the fastprep bead for 1 minute to homogenise the sample and then centrifuged at 11000 g for 2 minutes. 150 µL buffer SL3 was added and vortexed for 5 seconds before being incubated for 5 minutes at 4°C. The samples were then centrifuged for 1 minute at 11000 g. 700 µL of supernatant was loaded onto a NucleoSpin Inhibitor Removal Column (red ring) in a Collection Tube (make sure to keep the flowthrough) and centrifuged for 1 minute at 11000 g. 250 µL buffer SB was added and vortexed for 5 seconds. A NucleoSpin Soil Column (green ring) was placed in a collection tube and 550 µL sample was loaded onto the column. This was centrifuged for 1 minute at 11000 g and the flowthrough was discarded. This step was repeated with the remaining sample and the column was returned back into the collection tube. 500 µL buffer SB was added to the NucleoSpin Soil Column, centrifuged for 30 seconds at 11000 g and then the flowthrough was discarded. This was repeated with 550 µL of buffer SW1 in order to wash the silica membrane. Then 700 µL of buffer SW2 was added to

the NucleoSpin Soil Column, centrifuged for 30 seconds at 11000 g and the flowthrough was discarded. This step was repeated. After the flowthrough was discarded, the column and collection tube were then centrifuged for 2 minutes at 11000 g to dry the column. The column was transferred to a new collection tube and 100 µL of buffer SE was added to the column. The lid was not closed and it was incubated for 1 minute at RT. Then the lid was closed and it was centrifuged for 30 seconds at 11000 g. The eluted DNA can be used in the downstream applications and it was stored at -20°C until analysis.

### **3.6.2 PCR analysis**

The CAS-1200 Robotic liquid handling system (Corbett Life Science, Australia) was used for setting up all the PCRs and real-time PCR was performed on a Rotor-Gene<sup>TM</sup> 6000 (Corbett Life Science). A series of 10 fold-dilutions of extracted DNA were used as described in Di *et al.* (2010a, 2014) to determine the amplification efficiency of each diluted sample. The amplification efficiencies observed are shown in Table 3.2 (Di *et al.*, 2014).

**Table 3.2: Primer pairs and PCR conditions used in real-time qPCR analysis (Di et al., 2014)**

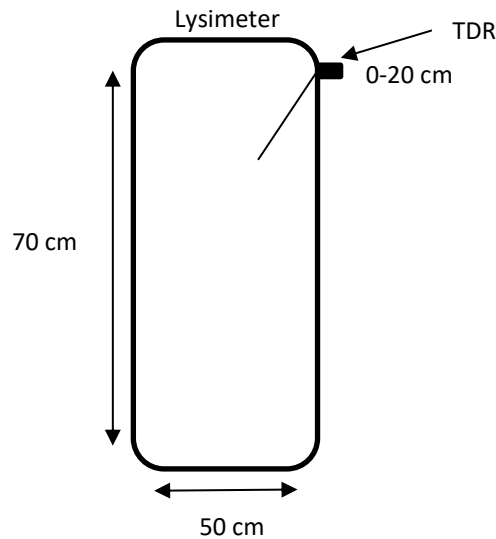
Target group	Primer name	Sequence (5' - 3')	Length of amplicon (bp)	Primer final concentration (nM)	Thermal profile	Amplification efficiency (R <sup>2</sup> > 0.99) (%)	References
Bacterial <i>amoA</i>	amoA1F amoA2R	5'-GGGGTTTCTACTGGTGGT-3' 5'-CCCCTCKGSAAAGCCTTCTTC-3'	491	250	95 °C for 2 min - x 1 cycle; 95 °C for 20 s, 57 °C for 30 s, 72 °C for 30 s, 85 °C for 15 s - x 40 cycles;	96-98	(Rotthauwe et al., 1997)
Archaeal <i>amoA</i>	Arch-amoAF Arch-amoAR	5'-STAATGGTCTGGCTTAGACG-3' 5'-GCGGCCATCCATCTGTATGT-3'	635	250	95 °C for 2 min - x 1 cycle; 95 °C for 20 s, 55 °C for 20 s, 72 °C for 30 s, 80 °C for 15 s - x 40 cycles;	92-94	(Francis et al., 2005)
<i>nirS</i>	cd3aF R3cd	5'-GTSAACGTSAAAGGARACSGG-3' 5'-GASTTCGGRTGSGTCTTGA-3'	410	750	95 °C for 2 min - x 1 cycle; 95 °C for 45 s, 55 °C for 45 s, 72 °C for 45 s, 85 °C for 20 s - x 40 cycles;	93-95	(Michotey et al., 2000) (Throback et al., 2004)
<i>nirK</i>	FlaCu R3Cu	5'-ATCATGGTCTGCCGCG-3' 5'-GCCTCGATCAGRTTGTGGTT-3'	474	780	95 °C for 2 min - x 1 cycle; 95 °C for 20 s, 63 °C for 30 s, 72 °C for 30 s, 85 °C for 15 s - x 40 cycles;	98-100	(Hallin and Lindgren, 1999)
<i>nosZ</i> (I)	nosZ-F nosZ1622R	5'-CGYTGTCMTGCAGCCAG-3' 5'-CGSACCTTSTTGCCSTYGCG-3'	424	750	95 °C for 2 min - x 1 cycle; 95 °C for 20 s, 58 °C for 30 s, 72 °C for 30 s, 85 °C for 15 s - x 40 cycles;	94-99	(Kloos et al., 2001) (Throback et al., 2004)
<i>nosZ</i> (II)	nosZ-II-F nosZ-II-R	5'-CTIGGICCIYTKCAYAC-3' 5'-GCIGARCARAAITCBGTRC-3'	698	1000	95 °C for 2 min - x 1 cycle; 95 °C for 30 s, 54 °C for 30 s, 72 °C for 40 s, 85 °C for 15 s - x 40 cycles;	76-81	(Jones et al., 2013)

The AOA and AOB *amoA* genes were quantified using the primers amoA1F/amoA2R and Arch-amoAF/Arch-amoAR as described by Di *et al.* (2010a, 2014). The PCR product specificity was confirmed after amplification using a melting curve analysis. This was carried out using the Rotor-Gene<sup>TM</sup> 6000 series software 1.7. Standard curves for real-time PCR assays (AOA and AOB *amoA* gene) were developed as described in Di *et al.* (2010a, 2014). This was used to determine the sample copy numbers (Table 3.2).

Additional soil samples for denitrifying microbial gene abundance were assessed at the same time as the samples taken for mineral N assays. The soil samples taken from all six treatments were analysed to determine the abundance of the NO<sub>2</sub><sup>-</sup> reductase genes *nirS* and *nirK*, and the N<sub>2</sub>O reductase gene *nosZ*. Using PCR conditions described earlier, each gene fragment was PCR-amplified using Premix Ex Taq<sup>TM</sup> (TaKaRa, Norrie Biotech, Auckland, New Zealand). A series of 10:1 dilutions of amplicon standards over a range of concentrations from 10<sup>1</sup> to 10<sup>7</sup> copies per microliter were used in generating standard curves used for each gene quantification (Di *et al.*, 2014; Treweek, 2015).

### **3.7 Soil moisture**

Volumetric soil moisture content was measured hourly for each lysimeter using time domain reflectometry (TDR) probe (Campbell Scientific water content Reflectometer, CS615) inserted into the soil column (encased in 50 cm x 70 cm lysimeter) between 0-20 cm depth (Fig 3.9). The daily average moisture content values were then calculated and recorded for the duration of the experiment. These data were later used to determine a relationship between soil moisture content and N<sub>2</sub>O emissions as described in section 4.1.2.3.



**Figure 3.9: TDR probe inserted into the soil column between 0-20 cm depth.**

In addition, gravimetric soil moisture content was determined each time soil sampling was done from the accompanied soil blocks (Method 3.4). To measure the soil moisture content, 14 g (approximately) of fresh soil was weighed in a paper cup, oven dried at 105°C for 24 hours and reweighed. The following equation was used to calculate the soil moisture (%):

$$\text{Soil moisture (\%)} = (\text{wet soil (g)} - \text{dry soil (g)}) / \text{dry soil (g)} \times 100 \quad \text{(Eqn. 4)}$$

Water-filled pore space (WFPS) was calculated as the ratio of the volumetric soil water content (SWC) to the total pore space (Saggar *et al.*, 2004). The formulae below was used to calculate the Total pore space:

$$\text{Total pore space (\%)} = 100[1 - (\text{bulk density} / \text{particle density})] \quad \text{(Eqn. 5)}$$

The soil bulk density was determined from undisturbed soil core samples taken from three lysimeters under the three irrigation systems (spray vs. roto-rainer vs. flood) with pasture growing on them. The particle density was assumed to be 2.65 Mg m<sup>-3</sup>. Total porosity (TP) was calculated for each soil under each irrigation system using equation 5 (Eqn. 5) and this is shown in Table 3.3.

**Table 3.3: Total porosity (TP)**

Irrigation	Bulk density dry (g/m <sup>3</sup> )	Total pore space (%)
Spray	1.37	48.15
Roto-rainer	1.36	48.59
Flood	1.39	47.60

From the table above, WFPS was calculated for each day under each irrigation system.

### 3.8 Climate data

Daily air temperature, daily soil temperature, daily rainfall and irrigation, and daily soil moisture for the trial period were collected from the Lincoln Met Station using the Vista Data Vision Database (<http://v-vision.lincoln.ac.nz/vdv/dashboard.php>).

### 3.9 Data and statistical analysis

Nitrous oxide emission rates were calculated from the increase in concentration of N<sub>2</sub>O between the first and second, and second and third gas samples taken at each sampling event (Hutchinson & Mosier, 1981). Daily N<sub>2</sub>O fluxes were then calculated on the assumption that the calculated hourly flux represented the average hourly flux for that day (de Klein *et al.*, 2003). Total N<sub>2</sub>O emissions were calculated by integrating the daily emission fluxes (Treweek *et al.*, 2016b). The emission factor (EF<sub>3</sub>) or the proportion of N emitted as N<sub>2</sub>O-N from urine-N applied was calculated using Equation (6) (de Klein *et al.*, 2003):

$$EF_3(\%) = \frac{N_2O-N \text{ total (urine)} - N_2O-N \text{ total (control)}}{Urine-N \text{ applied}} \times 100 \quad (\text{Eqn. 6})$$

where EF<sub>3</sub>(%) is the emission factor, 'N<sub>2</sub>O-N total (urine)' is the cumulative total N<sub>2</sub>O-N emitted (kg N<sub>2</sub>O-N/ha) from a urine treatment, 'N<sub>2</sub>O-N total (control)' is the cumulative total N<sub>2</sub>O emitted (kg N<sub>2</sub>O-N/ha) from the comparative no-urine treatment, and 'Urine-N applied' is the amount of N added as urine (kg N/ha).

The mean values and standard error of the means for daily and total N<sub>2</sub>O emissions, AOB and AOA community abundance, NH<sub>4</sub><sup>+</sup> concentration, NO<sub>3</sub><sup>-</sup> concentration and abundance of gene copy numbers for the denitrifiers were calculated based on the four replicates for each treatment using Microsoft Excel 2013 (Microsoft Corporation, USA). Least significant differences and p values were calculated using two way analysis of variance in Genstat® (Version 16.1, VSN International Ltd, UK). Total N<sub>2</sub>O values were log-transformed to ensure homogeneity of residual errors in order to determine the differences between the irrigation

systems. The daily N<sub>2</sub>O emissions were log<sub>10</sub>-transformed to determine its relationship with SWC, WFPS and temperature using Microsoft Excel 2013 (Microsoft Corporation, USA). The regression analysis for these factors (SWC, WFPS & temperature) were calculated using Microsoft Excel 2013.



## Chapter 4

### The effect of different irrigation systems on N<sub>2</sub>O emissions, soil nitrifiers and denitrifiers.

#### 4.1 Results

##### 4.1.1 Climate

During the experimental period (15<sup>th</sup> February to 28<sup>th</sup> June 2016), the daily average air temperature varied from a high of 25.5°C on the 26<sup>th</sup> of February 2016 (summer) to a low of 4.6°C on the 2<sup>nd</sup> of June 2016 (winter) (Fig 4.1). The daily average soil temperature varied from a high of 22°C in February (summer) to a low of 5.8°C on the 7<sup>th</sup> of June (winter) (Fig 4.2).

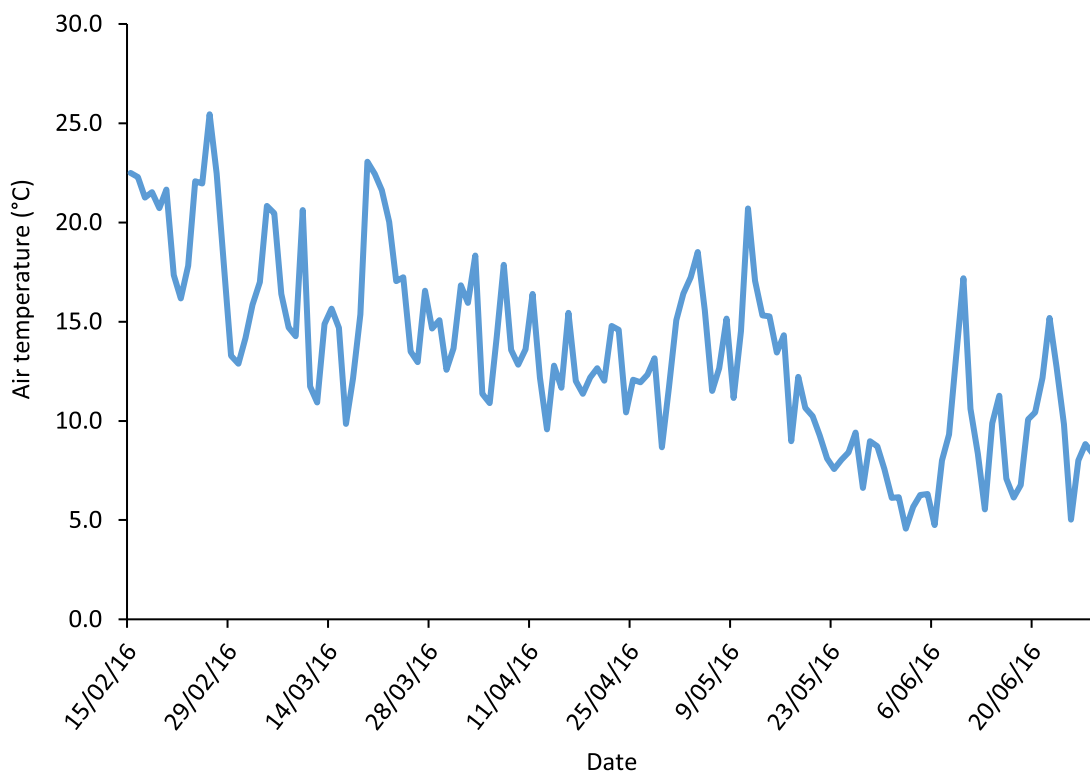
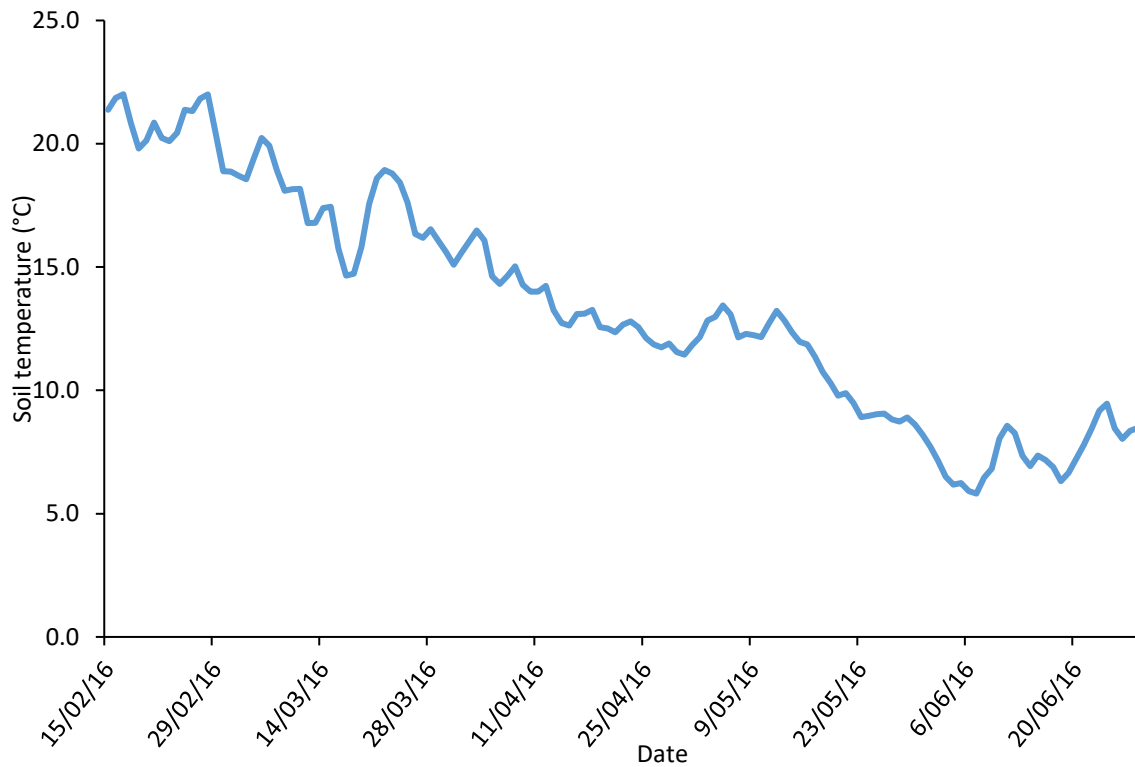
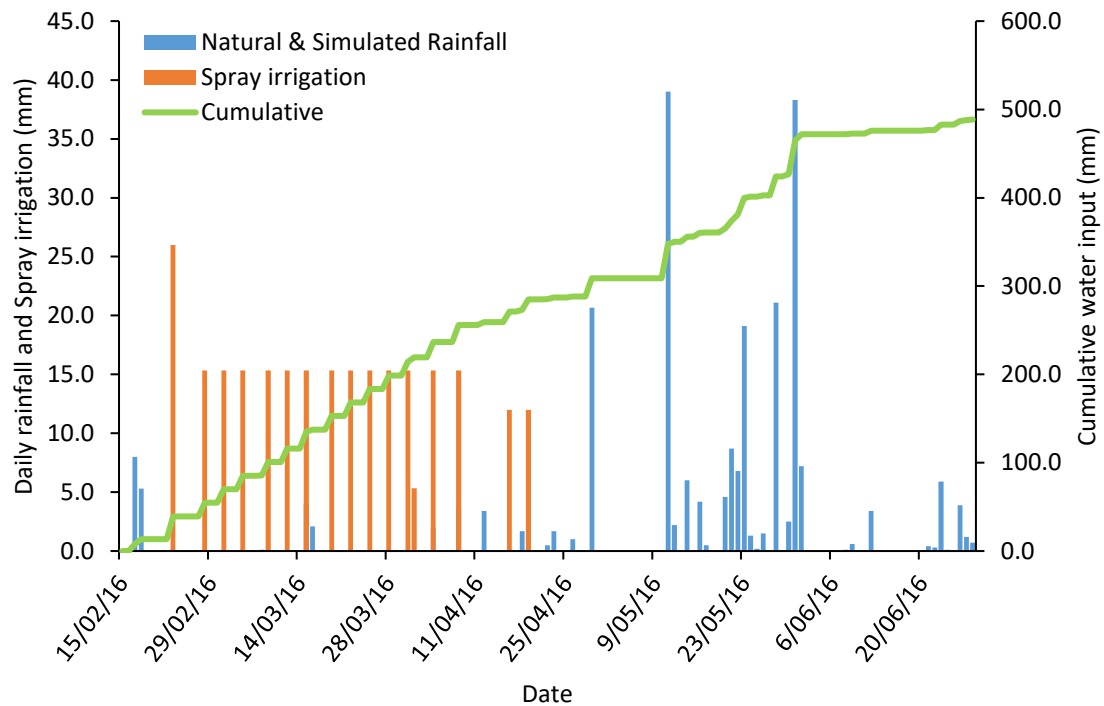


Figure 4.1: Daily average air temperature.

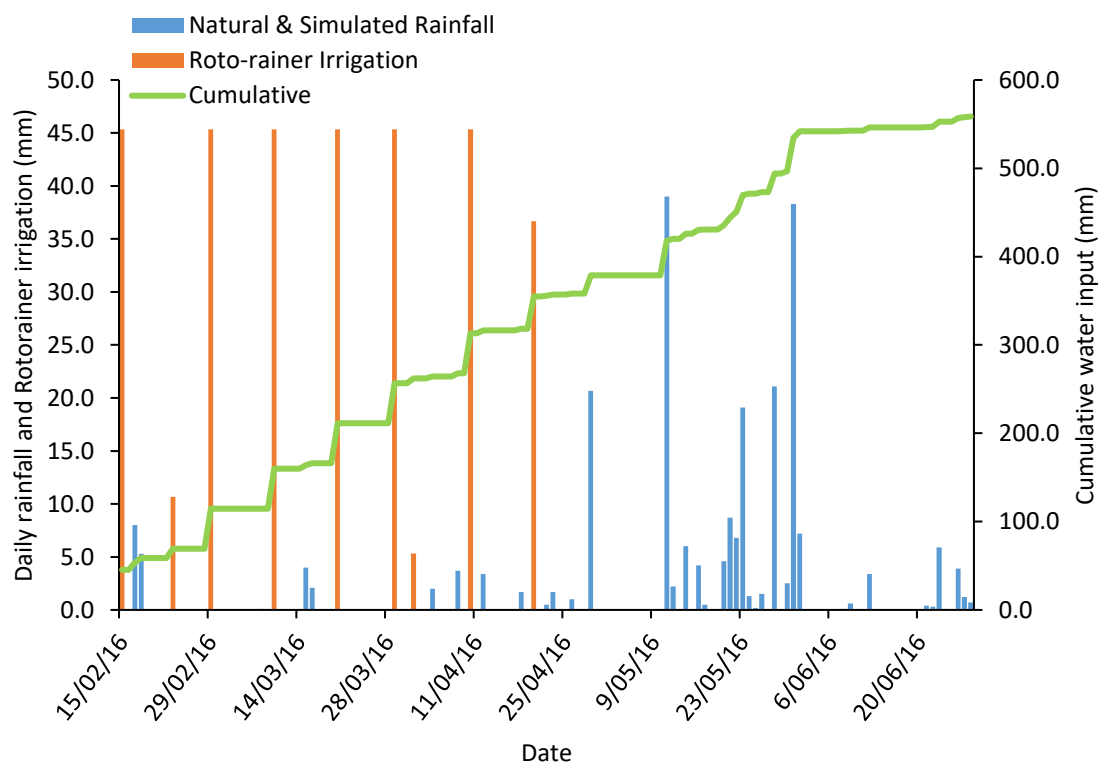


**Figure 4.2: Daily average soil temperature**

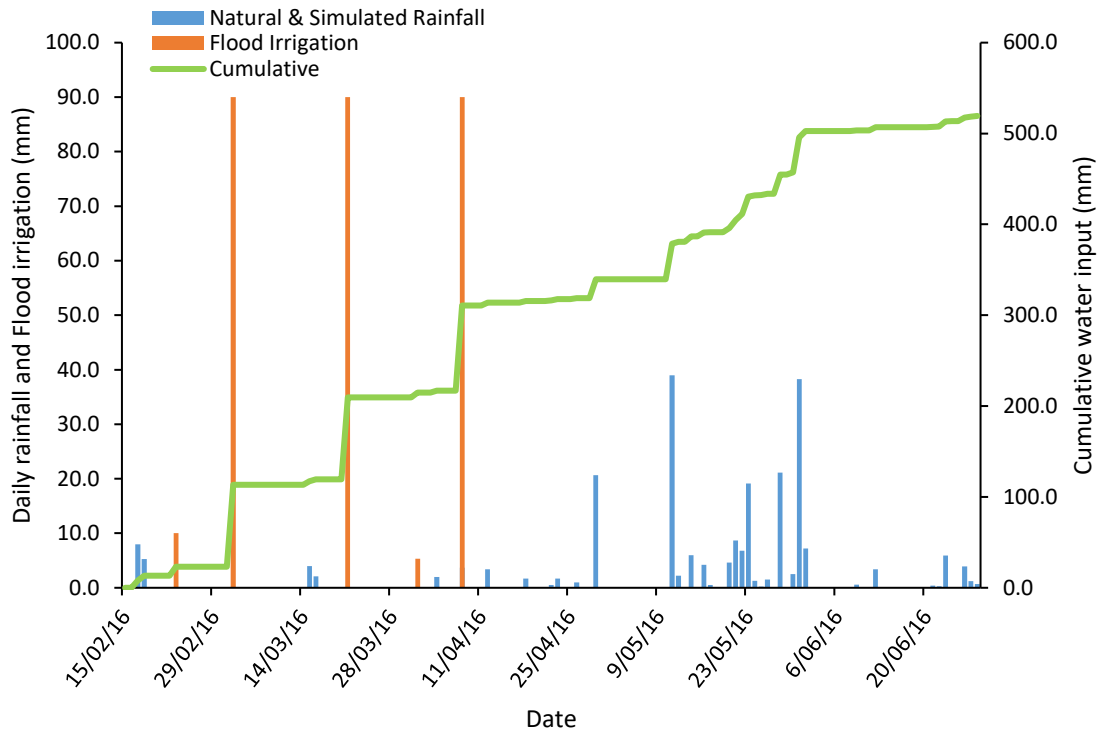
Natural rainfall, simulated rainfall and irrigation were applied to the soil. The effects of the three irrigation systems were tested: spray, roto-rainer and flood as described in Table 3.1 in the Materials and Methods chapter. For the spray irrigation, the total water input was 488.6 mm. This included 234 mm rainfall (natural and simulated) and 254.6 mm spray irrigation (Fig 4.3). For the roto-rainer irrigation, the total water input was 558.7 mm. This included 234 mm rainfall (natural and simulated) and 324.7 mm roto-rainer irrigation (Fig 4.4). For the flood irrigation, the total water input was 519.3 mm. This included 234 mm rainfall (natural and simulated) and 285.3 mm flood irrigation (Fig 4.5).



**Figure 4.3: Daily rainfall, irrigation and cumulative water inputs (Spray irrigation).**

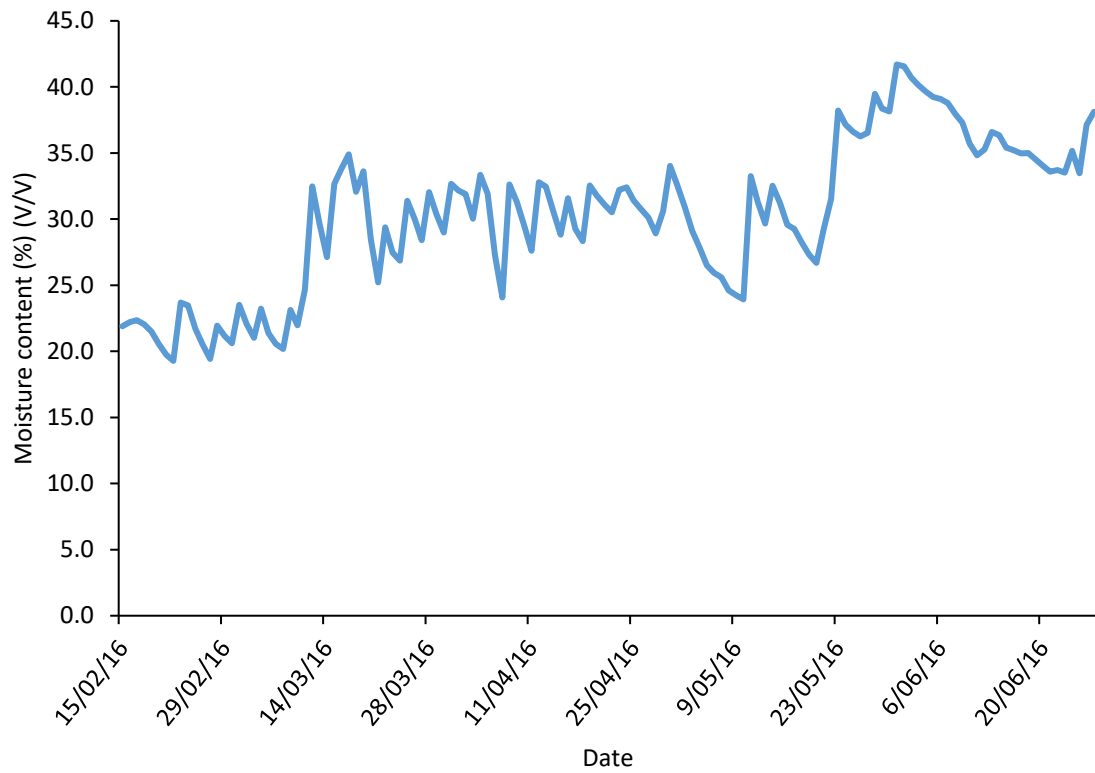


**Figure 4.4: Daily rainfall, irrigation and cumulative water inputs (Roto-rainer irrigation).**

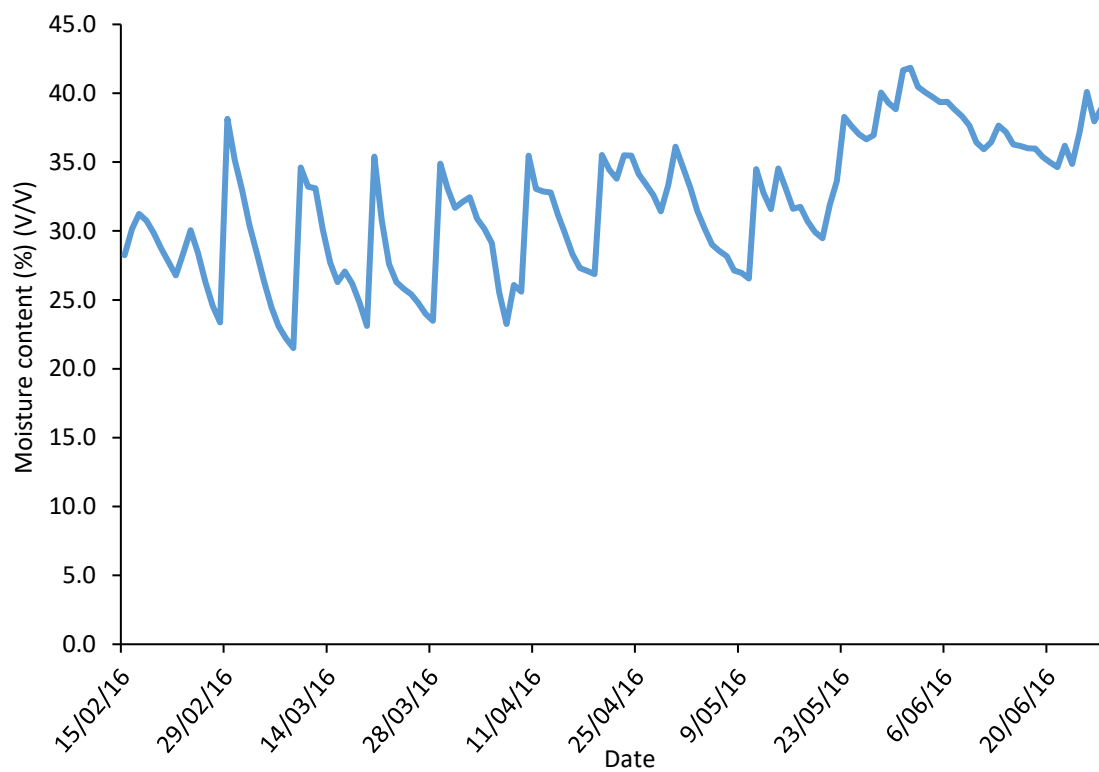


**Figure 4.5: Daily rainfall, irrigation and cumulative water inputs (Flood irrigation).**

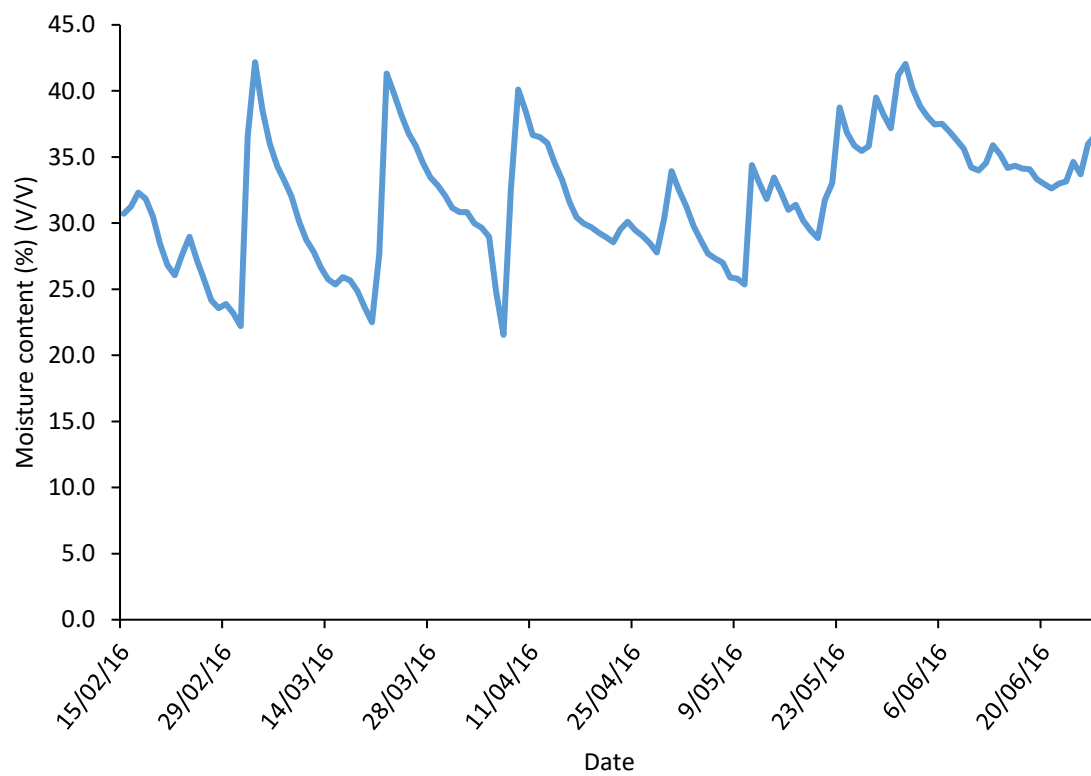
Soil moisture content responded to the rainfall and irrigation inputs with peaks in soil moisture detected after the rainfall occurred or irrigation water was applied (Fig 4.6, Fig 4.7, and Fig 4.8). For the spray irrigation, there were rapid small decreases and increases in moisture content with a maximum of 41.7 % moisture content on the 31<sup>st</sup> of May and a minimum of 19.3 % moisture content on the 22<sup>nd</sup> of February (Fig 4.6). Under the roto-rainer irrigation, there were more gradual peaks and troughs in the moisture content with a maximum of 41.9 % moisture content on the 1<sup>st</sup> of June and a minimum of 21.5 % moisture content on the 9<sup>th</sup> of March (Fig 4.7). For the flood irrigation, there were larger peaks and troughs in the moisture content with a maximum of 42.2 % moisture content on the 4<sup>th</sup> of March and a minimum of 21.6 % moisture content on the 7<sup>th</sup> of April (Fig 4.8).



**Figure 4.6: Daily average soil moisture (Spray irrigation).**



**Figure 4.7: Daily average soil moisture (Roto-rainier irrigation).**

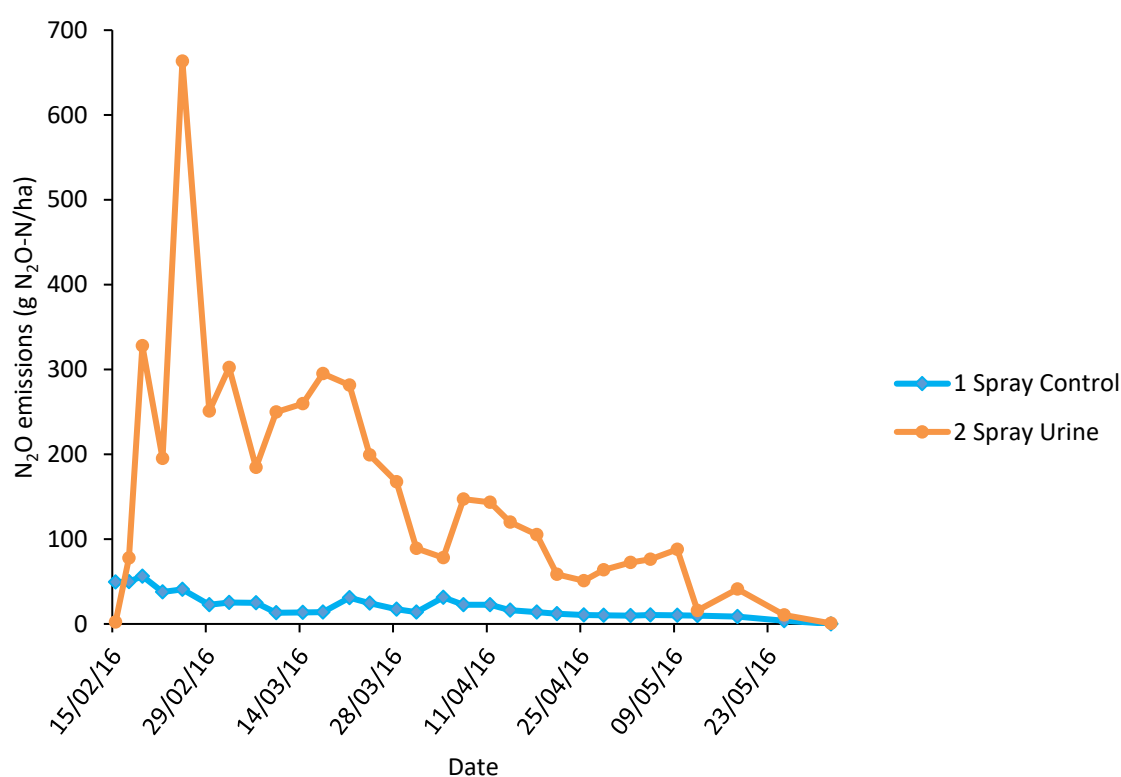


**Figure 4.8: Daily average soil moisture (Flood irrigation).**

## 4.1.2 N<sub>2</sub>O emissions

### 4.1.2.1 Daily N<sub>2</sub>O emissions

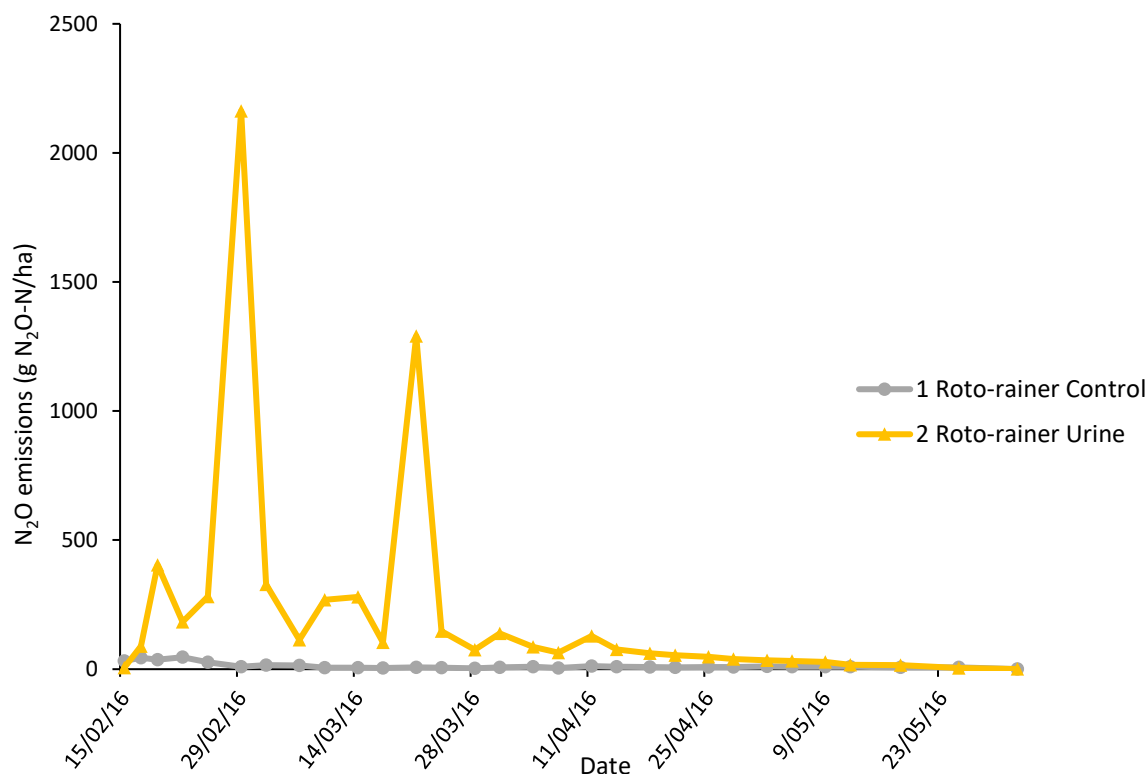
For the urine treatment under the spray irrigation, there was a small peak in the N<sub>2</sub>O-N flux (328 g N<sub>2</sub>O-N/ha) on the 19<sup>th</sup> of February (Fig 4.9). This was probably the result of the impact of a rainfall event which occurred on the 17<sup>th</sup> of February (Fig 4.3). After the small peak, another larger peak of 663 g N<sub>2</sub>O-N/ha was recorded on the 25<sup>th</sup> of February, after 26 mm of spray irrigation had been applied on the 23<sup>rd</sup> of February (Fig 4.9). After this highest peak, there was a gradual decline in the N<sub>2</sub>O-N flux. However, it did not reach background levels until the end of May due to the constant input of spray irrigation and rainfall (Fig 4.3, Fig 4.9).



**Figure 4.9: Daily N<sub>2</sub>O emissions (Spray irrigation).**

For the urine treatment under the roto-rainer irrigation, there was a small peak in the N<sub>2</sub>O-N flux (404 g N<sub>2</sub>O-N/ha) on the 19<sup>th</sup> of February (Fig 4.10). This was probably the result of the impact of irrigation applied on the 15<sup>th</sup> and rainfall on the 17<sup>th</sup> of February (Fig 4.4). After the small peak, the N<sub>2</sub>O-N flux reached the highest peak of 2162 g N<sub>2</sub>O-N/ha on the 29<sup>th</sup> of February (Fig 4.10). On the same day, 45.3 mm of irrigation was applied. There was also 10.7 mm of irrigation applied on the 23<sup>rd</sup> of February. On the 20<sup>th</sup> of March, 45.3 mm of irrigation was applied and the N<sub>2</sub>O-N flux reached a peak of 1291 g N<sub>2</sub>O-N/ha on the 21<sup>st</sup> of March (a day after

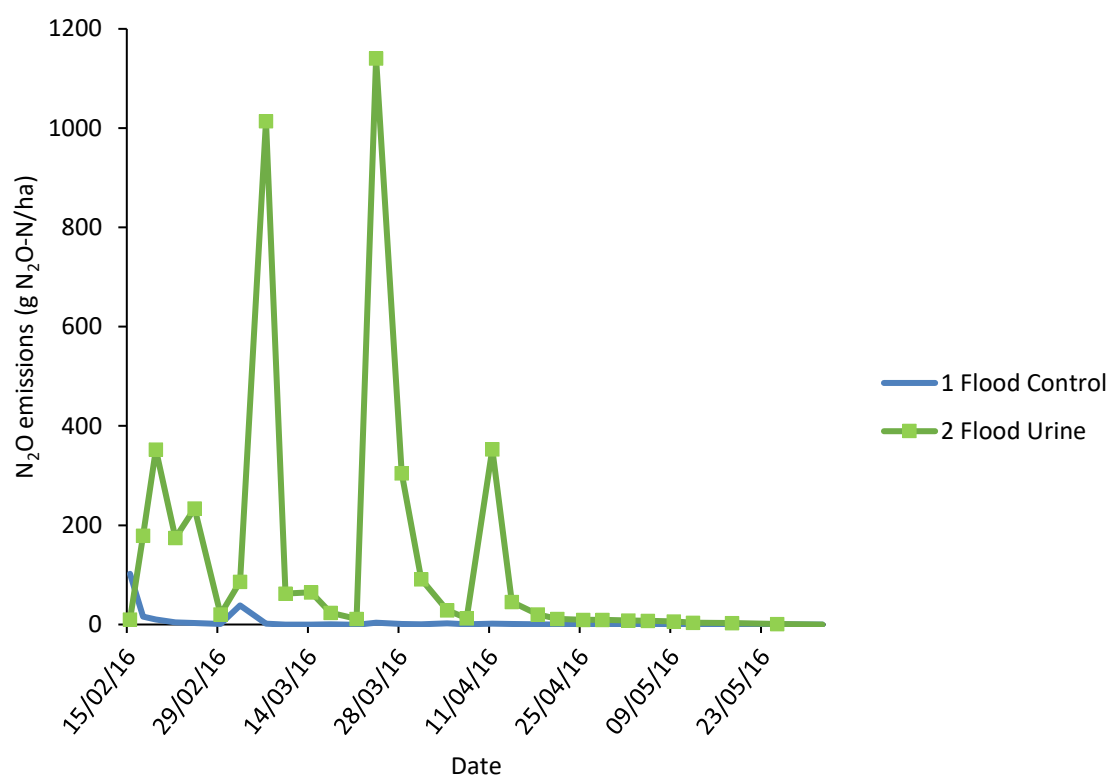
irrigation) (Fig 4.4, Fig 4.10). After the peak on the 21<sup>st</sup> of March, the N<sub>2</sub>O-N flux gradually declined close to background levels. Although the roto-rainer irrigation continued to be applied there were no more major peaks after the 11<sup>th</sup> of April.



**Figure 4.10: Daily N<sub>2</sub>O emissions (Roto-rainer irrigation).**

For the urine treatment under the flood irrigation, there was a small peak in the N<sub>2</sub>O-N flux (352 g N<sub>2</sub>O-N/ha) on the 19<sup>th</sup> of February (Fig 4.11). This can be attributed to the rainfall on the 17<sup>th</sup> and 18<sup>th</sup> of February which caused the soil moisture content to increase to 32% (Fig 4.5, Fig 4.8). On the 3<sup>rd</sup> of March, 90 mm of irrigation was applied and the N<sub>2</sub>O-N flux reached a peak of 1014 g N/ha on the 7<sup>th</sup> of March (4 days after irrigation). After that peak, there was a decline in the N<sub>2</sub>O-N flux until the 21<sup>st</sup> of March, when 90 mm of irrigation was applied and the N<sub>2</sub>O-N flux reached another peak of 1140 g N<sub>2</sub>O-N/ha on the 24<sup>th</sup> of March (3 days after irrigation). After that peak, the N<sub>2</sub>O-N flux declined. On the 8<sup>th</sup> of April, 90 mm of irrigation was applied and the N<sub>2</sub>O-N flux reached a small peak of 353g N<sub>2</sub>O-N/ha on the 11<sup>th</sup> of April (3 days after irrigation). After the small peak, the N<sub>2</sub>O-N flux gradually declined to background levels even though flood irrigation continued and there were no more N<sub>2</sub>O peaks after 11<sup>th</sup> of April (Fig 4.5, Fig 4.11).

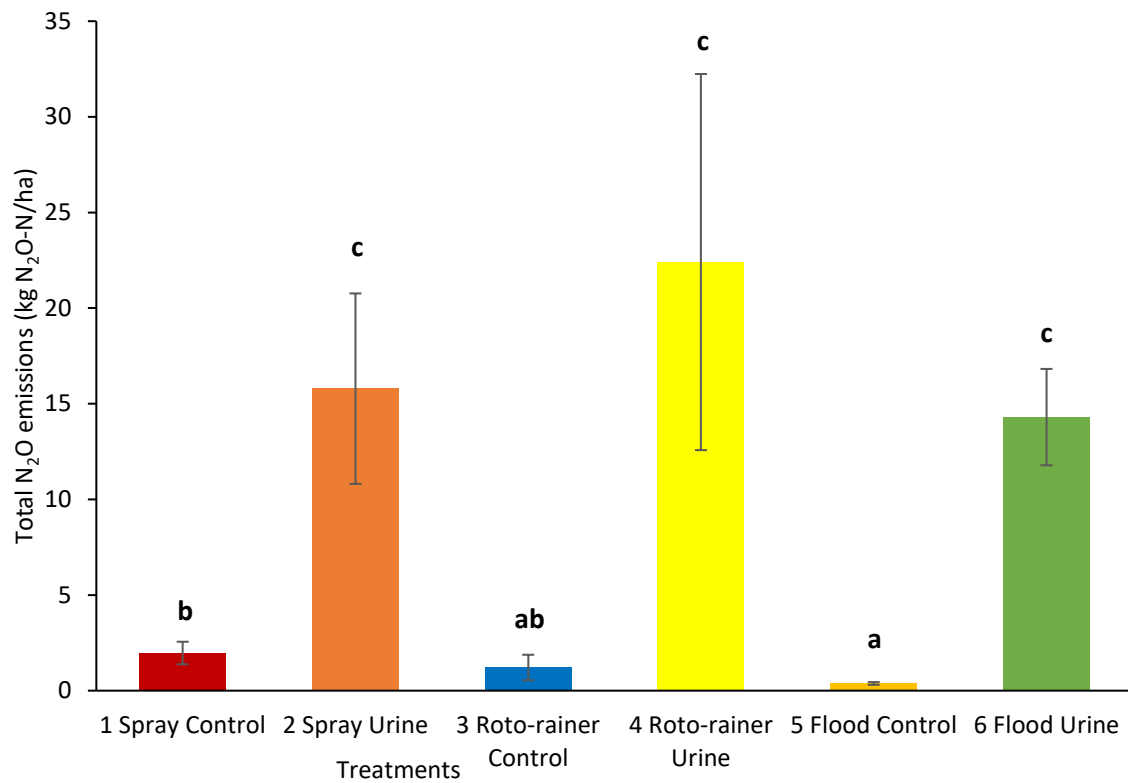




**Figure 4.11: Daily N<sub>2</sub>O emissions (Flood irrigation).**

#### 4.1.2.2 Total N<sub>2</sub>O emissions

Statistical analysis showed that, under the control treatments (i.e. no urine applied) for each irrigation system, there was no significant difference in N<sub>2</sub>O emissions. For the urine treatments, spray irrigation had a total N<sub>2</sub>O emission of 16 kg N<sub>2</sub>O-N/ha, roto-rainer irrigation had a total N<sub>2</sub>O emission of 22 kg N<sub>2</sub>O-N/ha and flood irrigation had a total N<sub>2</sub>O emission of 14 kg N<sub>2</sub>O-N/ha (Fig 4.12, Table 4.1). There was no significant difference between irrigation systems when urine was applied. However, the application of urine significantly ( $p < 0.05$ ) increased the N<sub>2</sub>O emissions compared to the non-urine (control) treatments.



**Figure 4.12: Total N<sub>2</sub>O emissions from spray, roto-rainer and flood irrigation systems with and without urine. Treatments with the same letter above the bars are not significantly different. The error bars indicate standard errors of the mean (SEM).**

The emission factor (EF<sub>3</sub>) values are shown in Table 4.1 below. The EF<sub>3</sub> were calculated using the formula derived from de Klein *et al.*, 2003 (Equation 6 in section 3.9). The EF<sub>3</sub> for this Templeton sandy loam soil were 1.97%, 3.03% and 1.99% under spray, roto-rainer and flood irrigation respectively (Table 4.1).

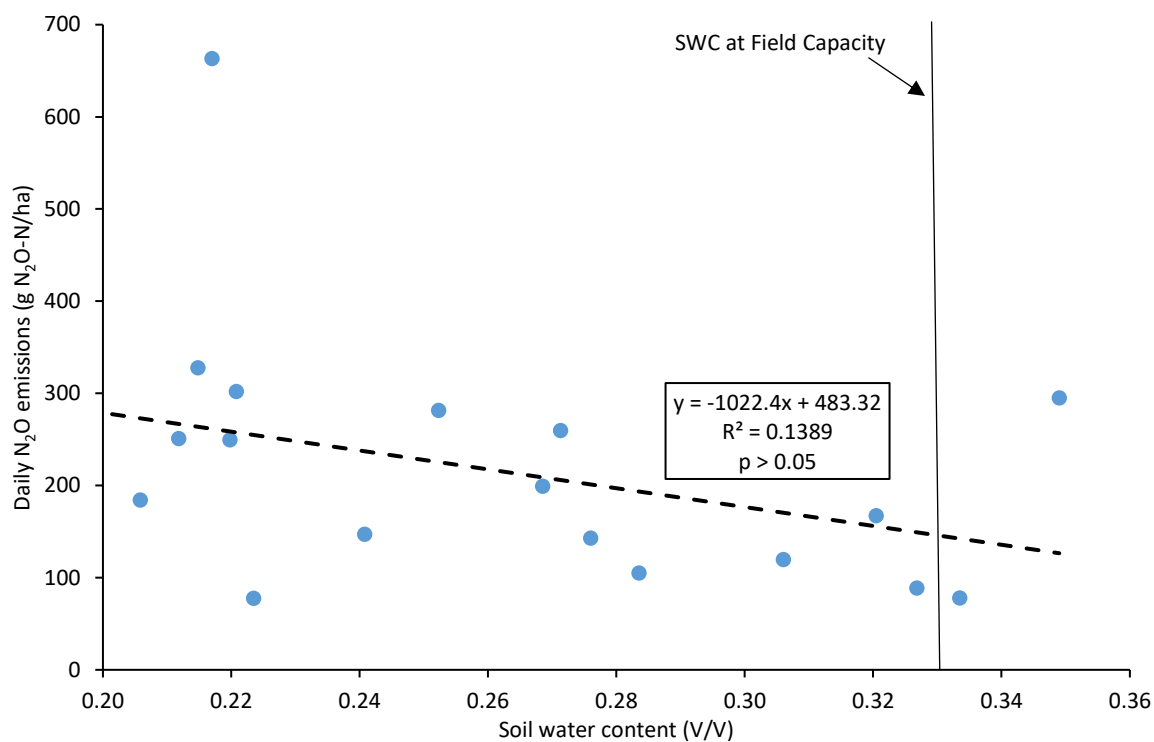
**Table 4.1: Total N<sub>2</sub>O emissions and proportion of applied N emitted as N<sub>2</sub>O (EF<sub>3</sub>) from the three irrigation systems (spray, roto-rainer and flood) under urine treatment.**

Type of irrigation	Total emissions (kg N <sub>2</sub> O-N/ha)		EF <sub>3</sub>	EF <sub>3</sub> (%)
	Total Urine	Total Control		
Spray	15.79 <sup>c</sup> (± 4.98)	1.97 <sup>b</sup> (± 0.59)	0.01974	1.97
Roto-rainer	22.41 <sup>c</sup> (± 9.83)	1.21 <sup>ab</sup> (± 0.67)	0.03029	3.03
Flood	14.30 <sup>c</sup> (± 2.52)	0.38 <sup>a</sup> (± 0.07)	0.01989	1.99

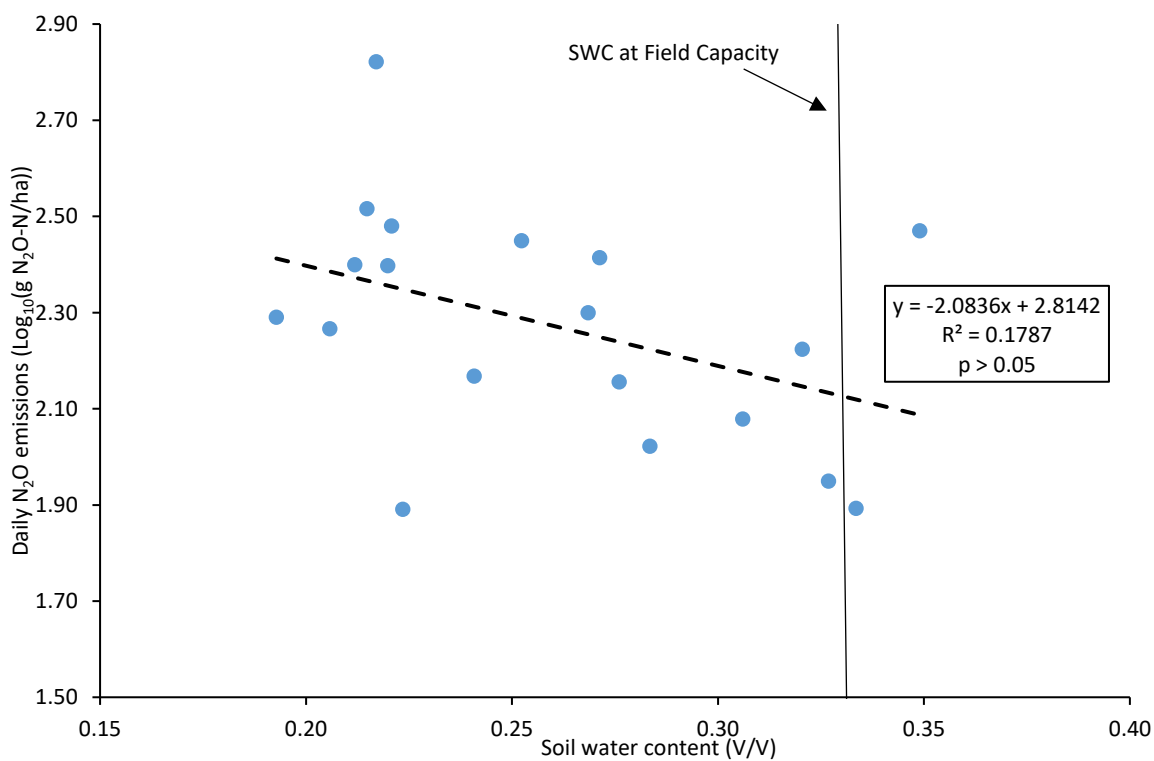
Same superscript letters indicate the treatments are not significantly different.

#### **4.1.2.3 Relationship between N<sub>2</sub>O emissions and Soil water content (SWC)**

Under the spray irrigation with urine treatment, the soil water content did not appear to be directly related to the N<sub>2</sub>O emissions. In fact, there was a weak negative correlation between soil water content and N<sub>2</sub>O emissions. The field capacity of the Templeton sandy loam soil in this experiment was 0.33 (V/V) (Appendix A) and the soil moisture content rarely exceeded field capacity (Fig 4.6). There was limited N<sub>2</sub>O emissions measured above field capacity (Fig 4.13). N<sub>2</sub>O emissions seemed to take place below the field capacity water content. There was a standout point on the graph (Fig 4.13) where N<sub>2</sub>O emission was 663.5 g N<sub>2</sub>O-N/ha when the SWC was 0.22. This might be a result of spray irrigation applied two days before. Before the N<sub>2</sub>O values were log<sub>10</sub>-transformed, the dataset for SWC, WFPS and soil temperature versus N<sub>2</sub>O emissions were truncated to include only the data when most N<sub>2</sub>O emissions took place (between 17<sup>th</sup> February and 18<sup>th</sup> April 2016). This was a result of the presence of NO<sub>3</sub><sup>-</sup> in the soil but, as NO<sub>3</sub><sup>-</sup> became limited in the soil after the 18<sup>th</sup> of April 2016 (Fig 4.44), N<sub>2</sub>O emissions decreased. The N<sub>2</sub>O emission values were log<sub>10</sub>-transformed to ensure homogeneity of residual errors in order to determine differences between treatments or to observe a clear relationship between SWC, WFPS, temperature and N<sub>2</sub>O emissions (Choudhary, *et al.*, 2002; Treweek *et al.*, 2016a). Figure 4.14 also showed a weak negative correlation between soil water content and N<sub>2</sub>O emissions.

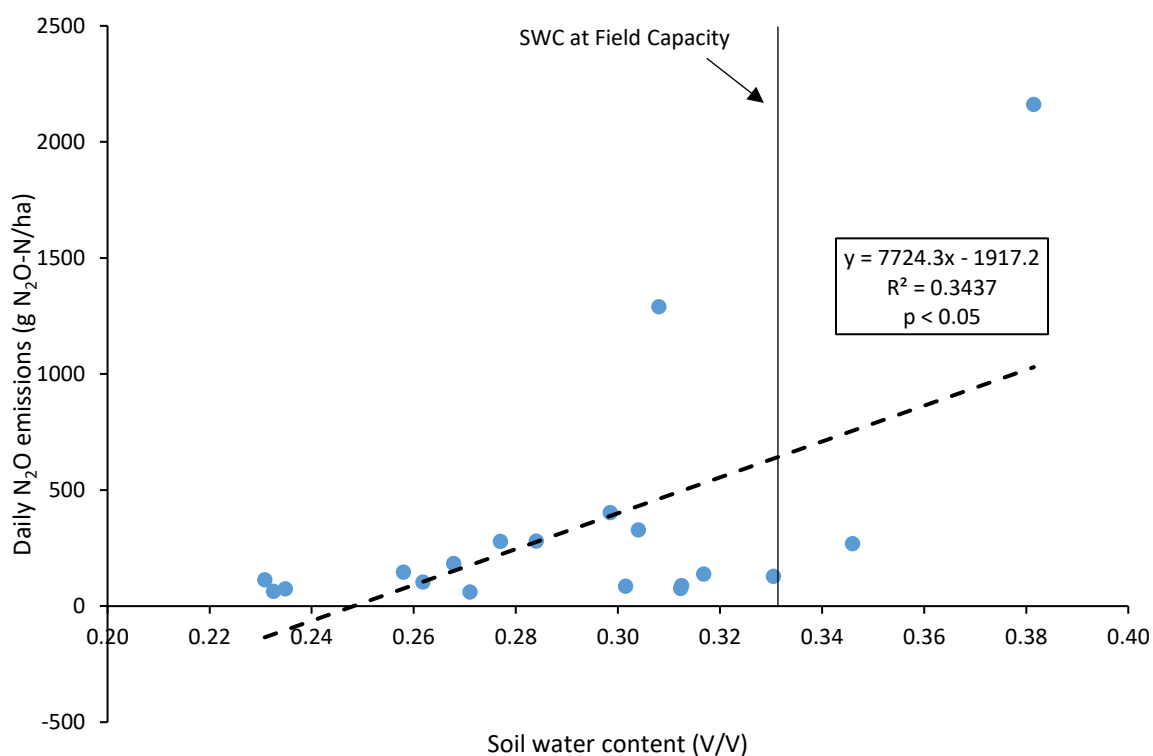


**Figure 4.13: The relationship between daily  $N_2O$  emissions and soil water content (SWC) under spray irrigation with urine treatment.**

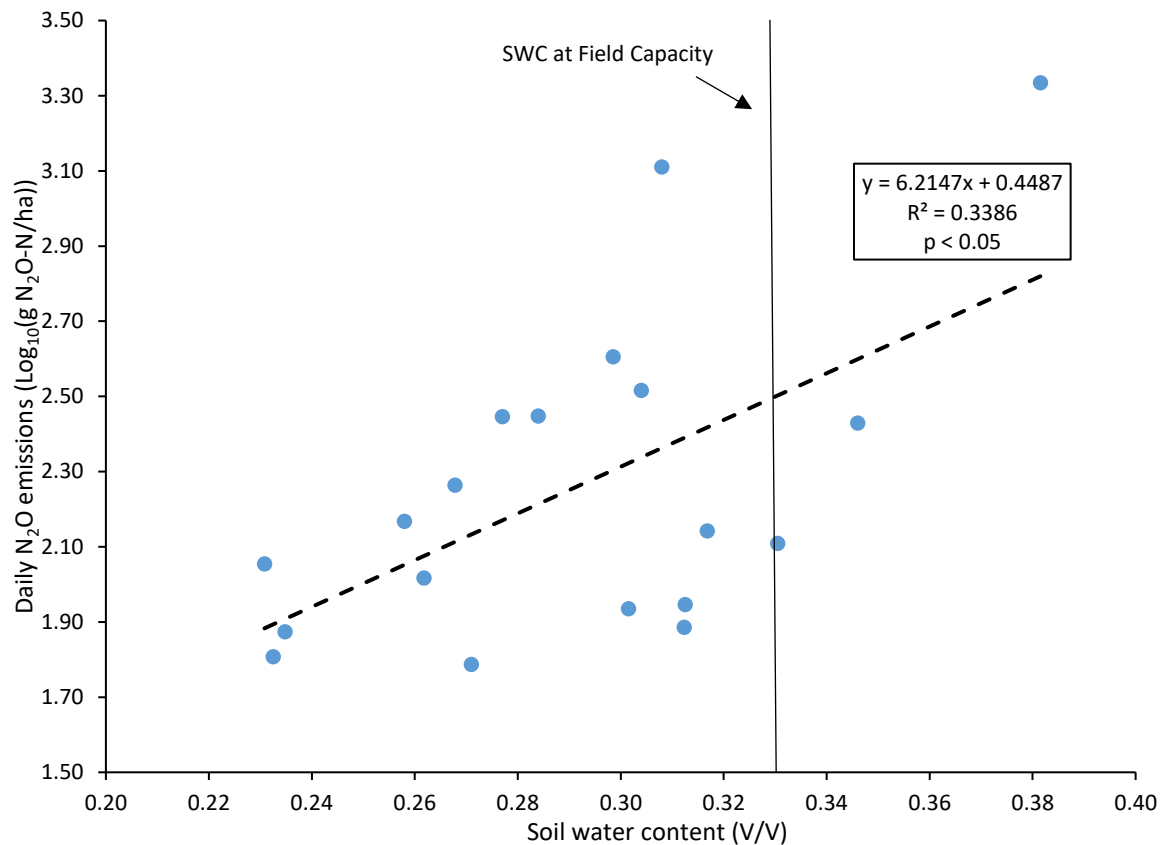


**Figure 4.14: The relationship between logarithmic transformed daily  $N_2O$  emissions and soil water content (SWC) under spray irrigation with urine treatment.**

Under the roto-rainer irrigation with urine treatment, the soil water content appeared to have a weak positive relationship with the N<sub>2</sub>O emissions (Fig 4.15). There were two standout points on the graph (Fig 4.15) where the N<sub>2</sub>O emission was 1291 g N<sub>2</sub>O-N/ha when the SWC was 0.31 and 2162 g N<sub>2</sub>O-N/ha when the SWC was 0.38. These might be a result of roto-rainer irrigation applied on the same day and a day before gas measurement, respectively. When the data were log<sub>10</sub>-transformed there was a stronger positive correlation between soil water content and N<sub>2</sub>O emissions (Fig 4.16). Most of the N<sub>2</sub>O emissions occurred below the SWC at field capacity.

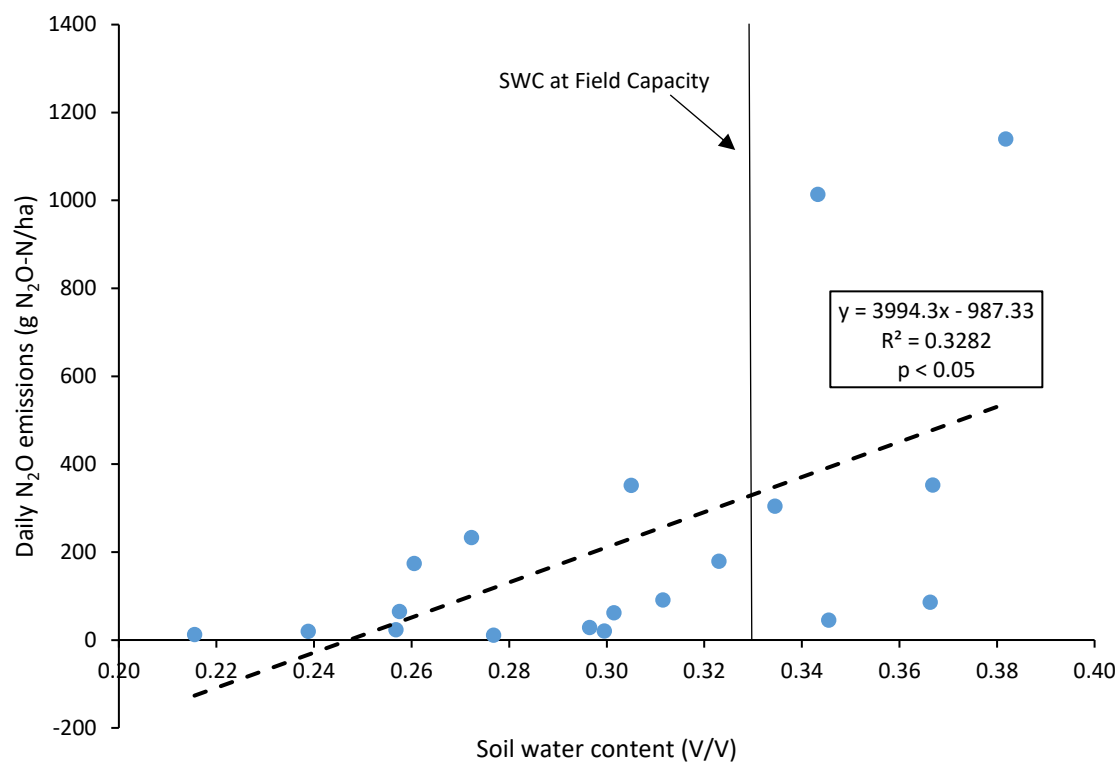


**Figure 4.15: The relationship between daily N<sub>2</sub>O emissions and soil water content (SWC) under roto-rainer irrigation with urine treatment.**

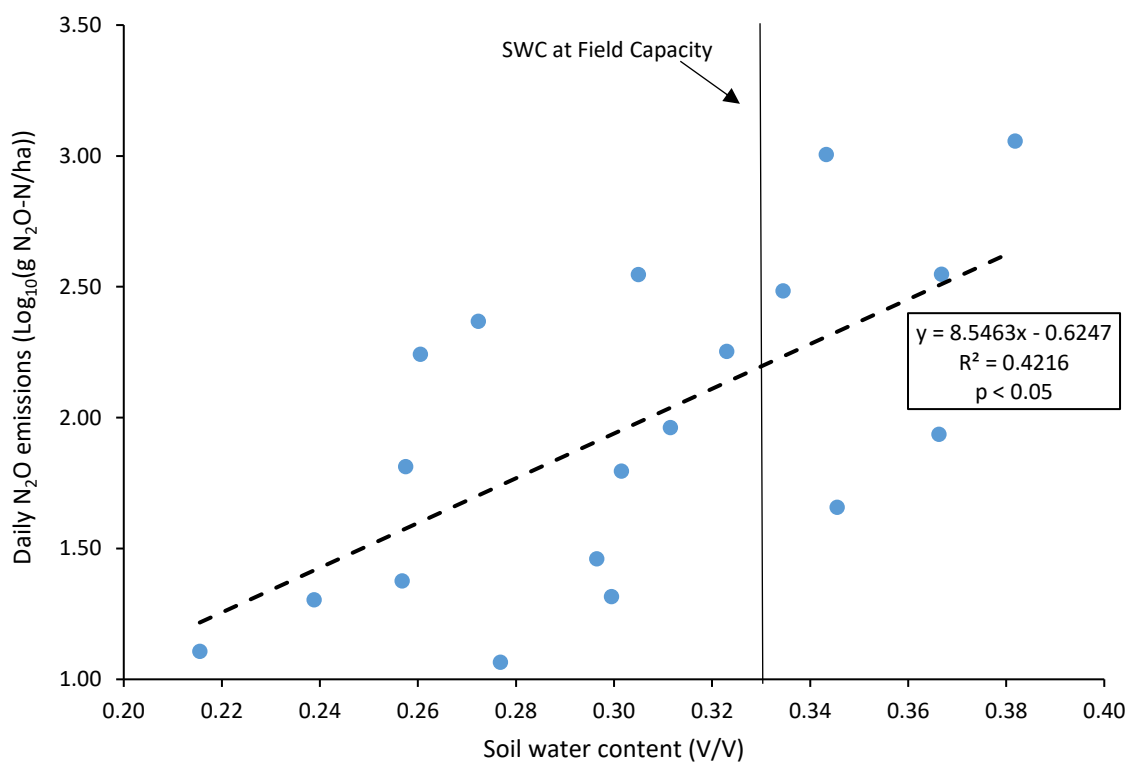


**Figure 4.16: The relationship between logarithmic transformed daily N<sub>2</sub>O emissions and soil water content (SWC) under roto-rainier irrigation with urine treatment.**

Under the flood irrigation with urine treatment, the soil water content appeared to have a weak positive relationship with the N<sub>2</sub>O emissions (Fig 4.17). The soil water content influenced N<sub>2</sub>O emissions. On the graph (Fig 4.17) there were also two standout points which showed N<sub>2</sub>O emissions being 1014 g N<sub>2</sub>O-N/ha when the SWC was 0.34 and 1140 g N<sub>2</sub>O-N/ha when the SWC was 0.38. These might be a result of flood irrigation applied 4 days and 3 days before gas measurement, respectively. When the N<sub>2</sub>O data were log<sub>10</sub>-transformed the soil water content was positively correlated to N<sub>2</sub>O emissions (Fig 4.18). Most of the N<sub>2</sub>O emissions occurred below the field capacity water content.

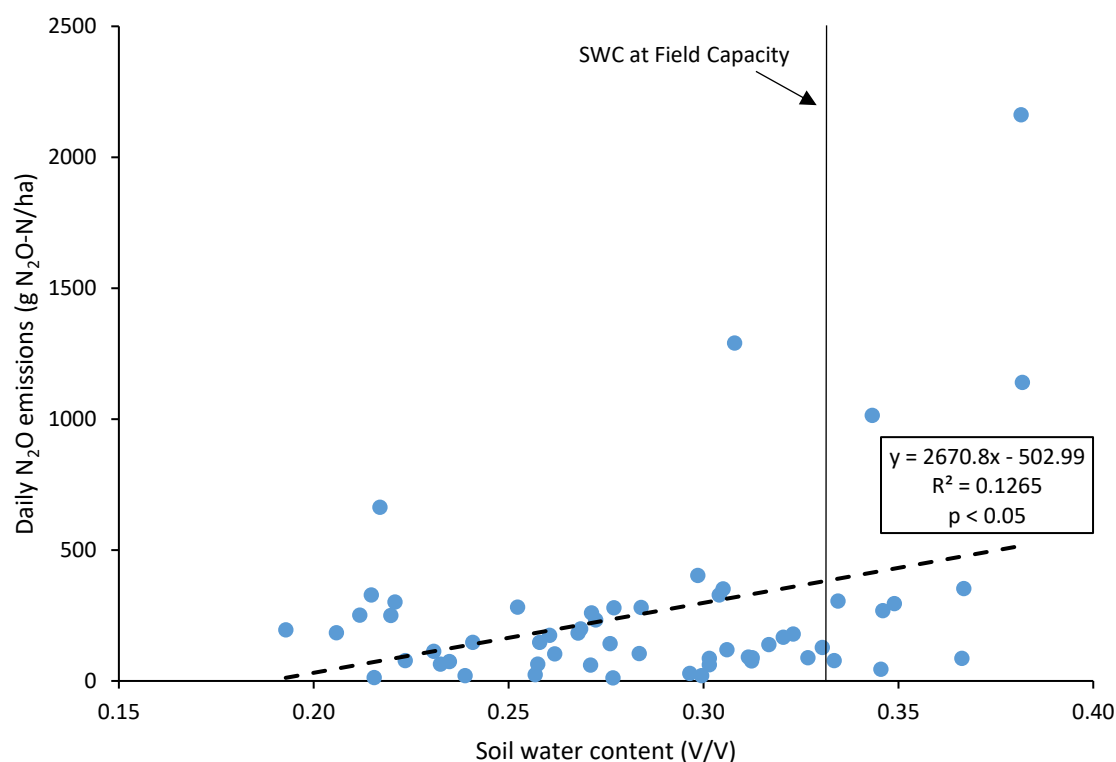


**Figure 4.17: The relationship between daily  $\text{N}_2\text{O}$  emissions and soil water content (SWC) under flood irrigation with urine treatment.**



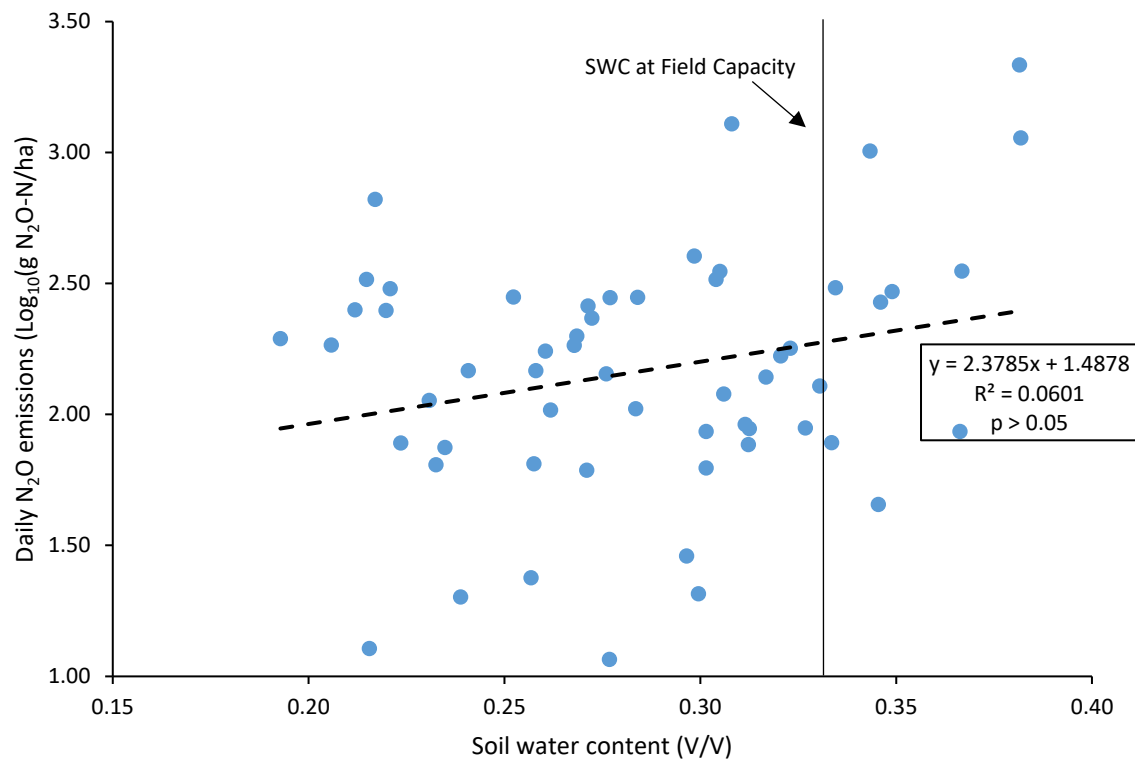
**Figure 4.18: The relationship between logarithmic transformed daily  $\text{N}_2\text{O}$  emissions and soil water content (SWC) under flood irrigation with urine treatment.**

Figures 4.19 and 4.20 showed the relationship between combined daily N<sub>2</sub>O emissions and SWC before log<sub>10</sub>-transformation and after log<sub>10</sub>-transformation of the data. Figure 4.19 showed a weak positive relationship between SWC and N<sub>2</sub>O emissions and most of the N<sub>2</sub>O emissions took place below the SWC at field capacity. After the data was log<sub>10</sub>-transformed, it did not improve the relationship; in fact, the relationship between SWC and N<sub>2</sub>O emissions was weaker (Fig 4.20).



**Figure 4.19: The relationship between combined daily N<sub>2</sub>O emissions and soil water content (SWC).**

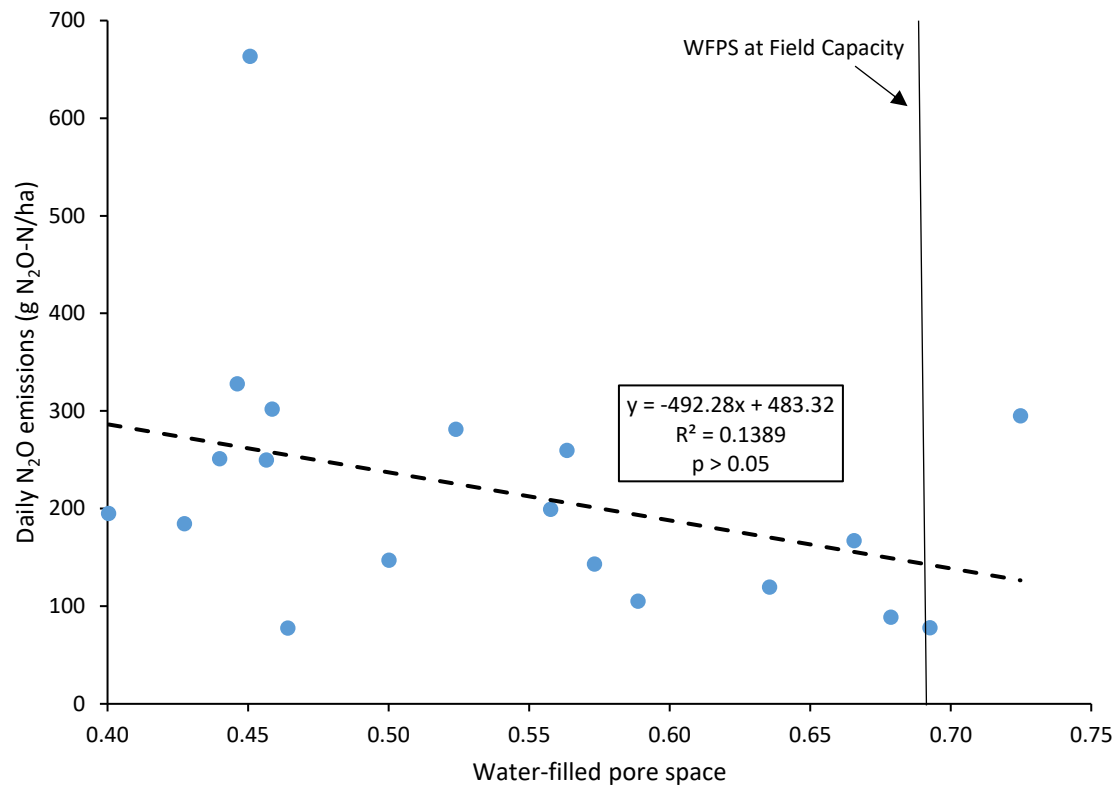




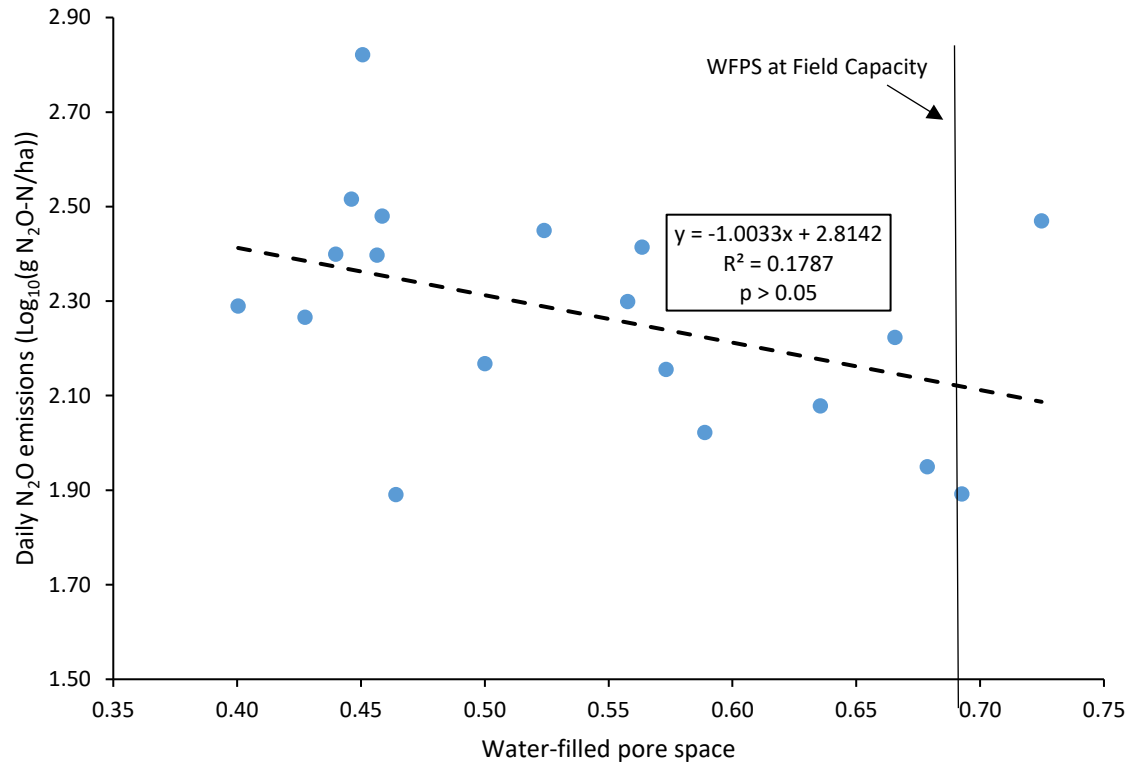
**Figure 4.20: The relationship between combined logarithmic transformed daily N<sub>2</sub>O emissions and soil water content (SWC).**

#### 4.1.2.4 Relationship between N<sub>2</sub>O emissions and Soil water-filled pore space (WFPS)

Soil water-filled pore space (WFPS) was used in this study because it normalises for differences in bulk density and particle density between soils (Saggar *et al.*, 2004a), and nitrification and denitrification rates have been reported to be closely related to the water-filled pore space (WFPS) of a soil (Sangar *et al.*, 2011). The results showed that, under the spray irrigation with urine treatment, WFPS did not have a strong relationship with the N<sub>2</sub>O emissions. There was a weak negative correlation between daily N<sub>2</sub>O emissions and the WFPS. The WFPS at field capacity was 0.69 for the Templeton sandy loam soil. There were few data and limited N<sub>2</sub>O emissions when the WFPS was above the field capacity WFPS (Fig 4.21). There was a standout point on the graph (Fig 4.21) where N<sub>2</sub>O emission was 663.5 g N<sub>2</sub>O-N/ha when the WFPS was 0.45. This might be a result of spray irrigation applied two days before. Figure 4.22 (Log<sub>10</sub>-transformed) also showed weak negative correlation between N<sub>2</sub>O emissions and the WFPS.

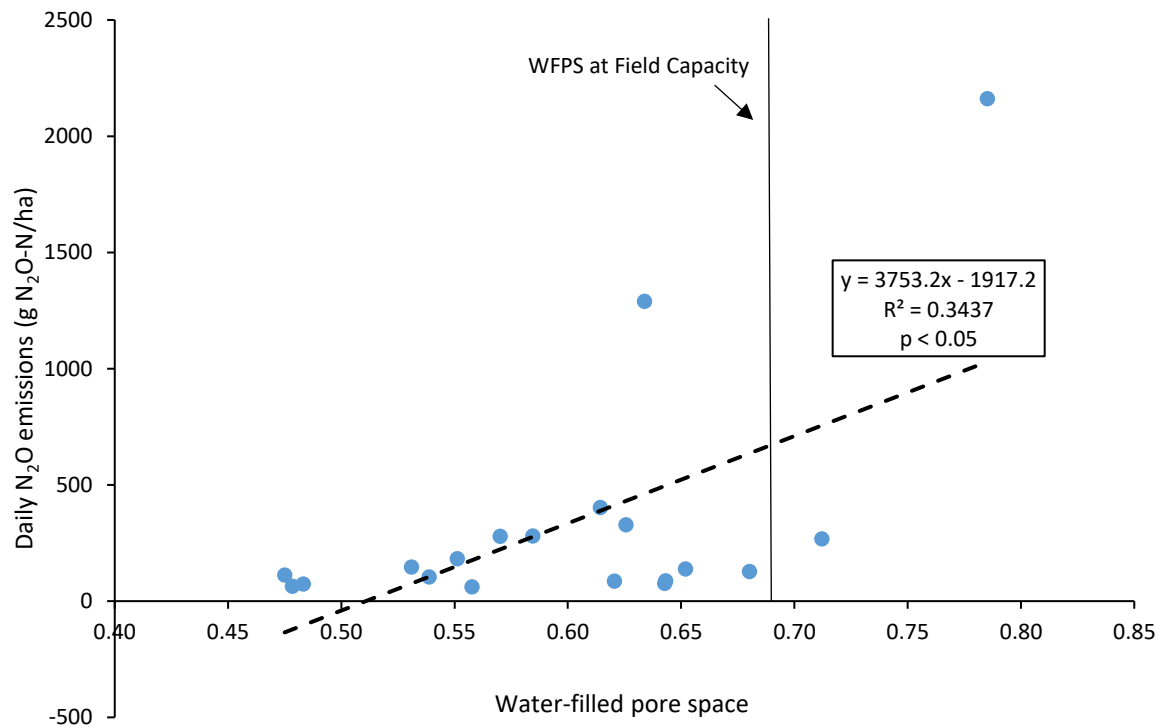


**Figure 4.21: The relationship between daily  $\text{N}_2\text{O}$  emissions and soil water-filled pore space (WFPS) under spray irrigation with urine treatment.**

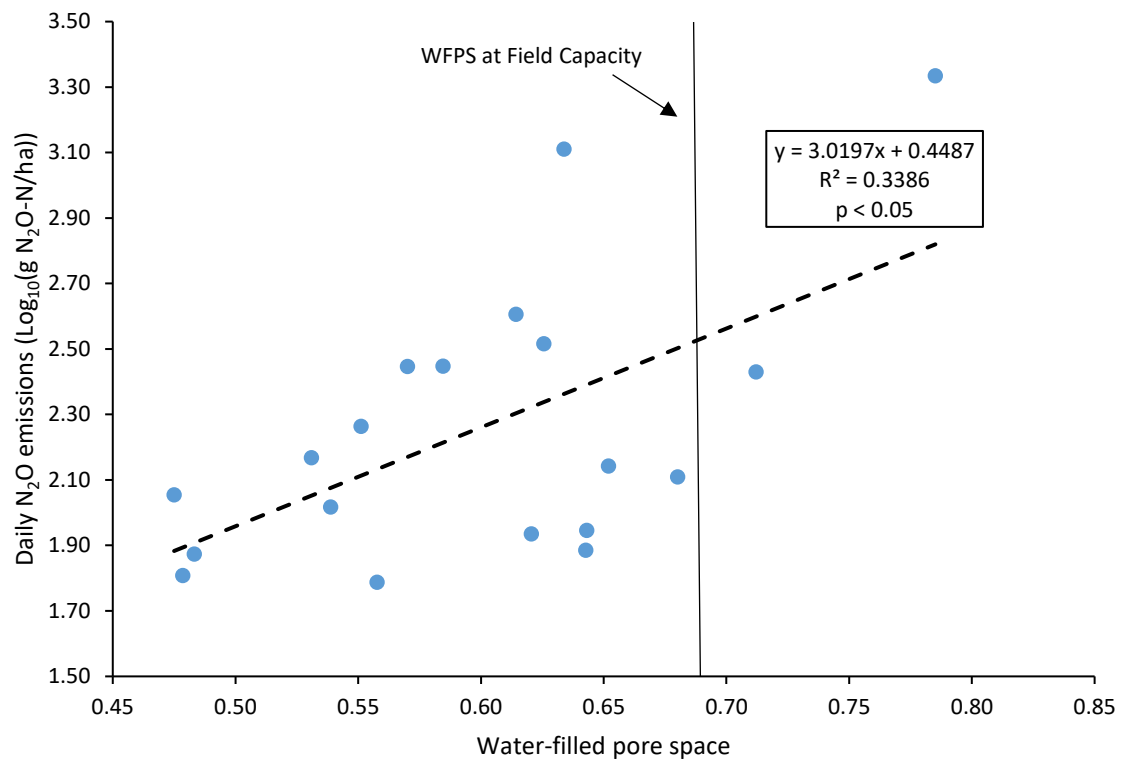


**Figure 4.22: The relationship between logarithmic transformed daily  $N_2O$  emissions and soil water-filled pore space (WFPS) under spray irrigation with urine treatment.**

The results showed that under the roto-rainer irrigation with urine treatment, WFPS appeared to have a weak positive correlation with the  $N_2O$  emissions. There was limited  $N_2O$  emission data when the WFPS was above field capacity (Fig 4.23). There were two standout points on the graph (Fig 4.23) where  $N_2O$  emission was 1291 g  $N_2O\text{-N/ha}$  when the WFPS was 0.63 and 2162 g  $N_2O\text{-N/ha}$  when the WFPS was 0.79. These might be a result of roto-rainer irrigation applied on the same day and a day before respectively. Figure 4.24 showed a clearer positive correlation between WFPS and  $N_2O$  emissions. Most of the  $N_2O$  emissions occurred below the field capacity WFPS.

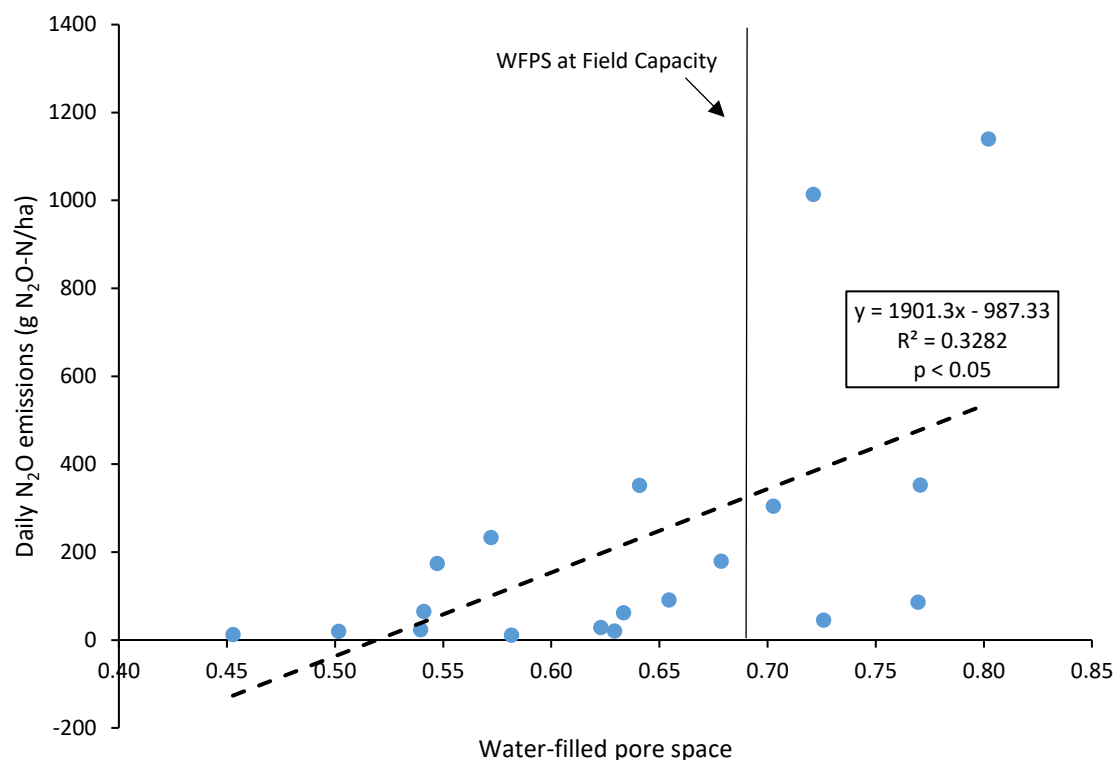


**Figure 4.23: The relationship between daily N<sub>2</sub>O emissions and soil water-filled pore space (WFPS) under roto-rainier irrigation with urine treatment.**

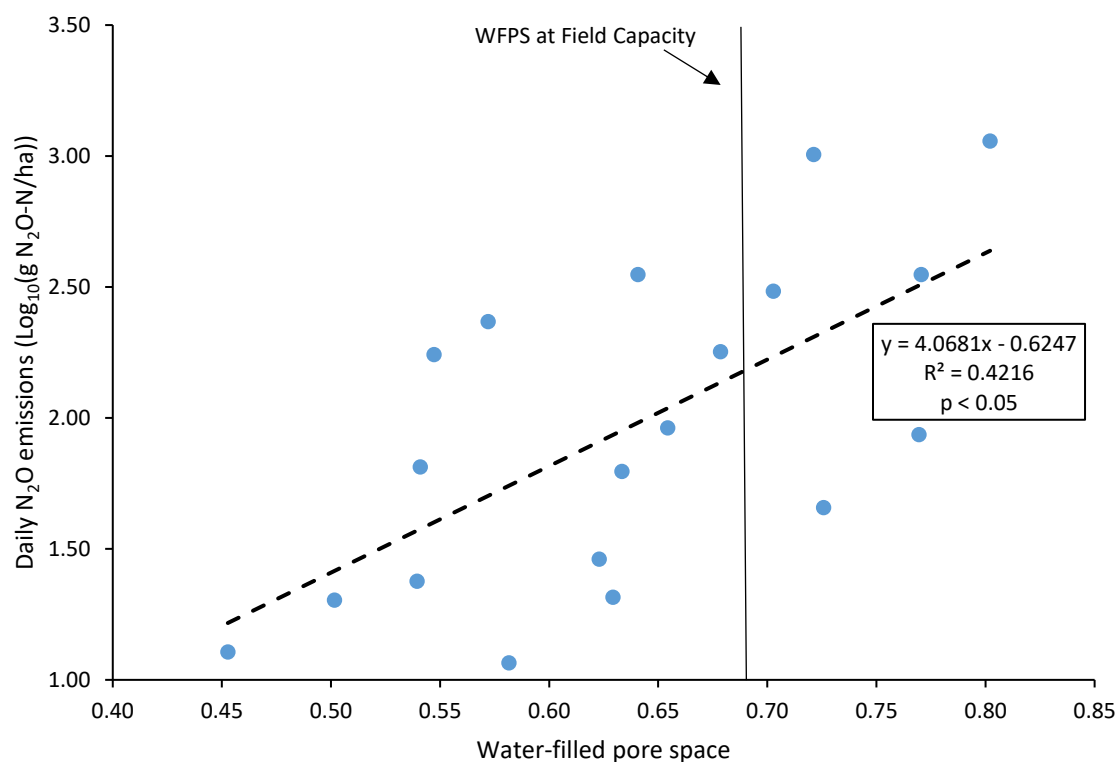


**Figure 4.24: The relationship between logarithmic transformed daily N<sub>2</sub>O emissions and soil water-filled pore space (WFPS) under roto-rainier irrigation with urine treatment.**

Under the flood irrigation with urine treatment, WFPS appeared to have a weak positive relationship with the N<sub>2</sub>O emissions (Fig 4.25). On the graph (Fig 4.25) there were two standout points which showed N<sub>2</sub>O emission was 1014 g N<sub>2</sub>O-N/ha when the WFPS was 0.72 and 1140 g N<sub>2</sub>O-N/ha when the WFPS was 0.80. These might be a result of flood irrigation applied 4 days and 3 days before respectively. Figure 4.26 showed that there was a positive correlation between WFPS and N<sub>2</sub>O emissions. Most of the N<sub>2</sub>O emissions occurred below the field capacity WFPS.

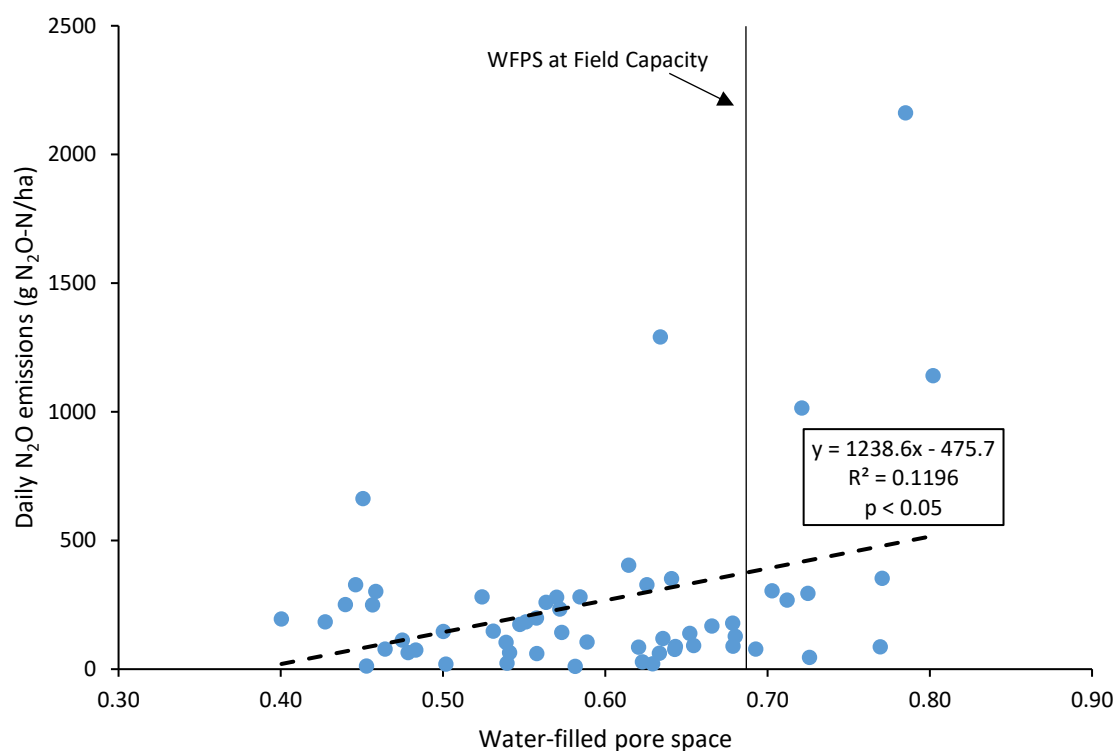


**Figure 4.25: The relationship between daily  $\text{N}_2\text{O}$  emissions and soil water-filled pore space (WFPS) under flood irrigation with urine treatment.**

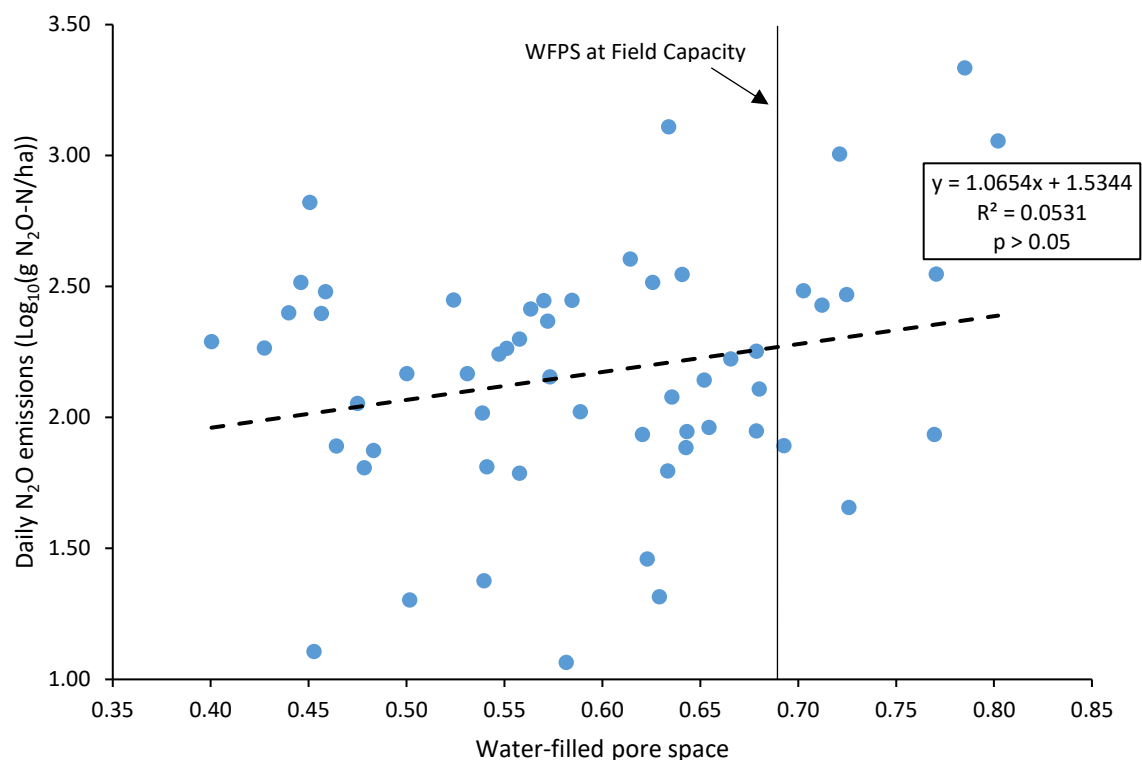


**Figure 4.26: The relationship between logarithmic transformed daily  $\text{N}_2\text{O}$  emissions and soil water-filled pore space (WFPS) under flood irrigation with urine treatment.**

Figures 4.27 and 4.28 showed the relationship between combined daily N<sub>2</sub>O emissions and WFPS before log<sub>10</sub>-transformation and after log<sub>10</sub>-transformation of the data. Figure 4.27 showed a weak positive relationship between WFPS and N<sub>2</sub>O emissions and most of the N<sub>2</sub>O emissions took place below the WFPS at field capacity. After the data was log<sub>10</sub>-transformed, it did not improve the relationship. The relationship between WFPS and N<sub>2</sub>O emissions was even weaker (Fig 4.28).



**Figure 4.27: The relationship between combined daily N<sub>2</sub>O emissions and water-filled pore space (WFPS).**

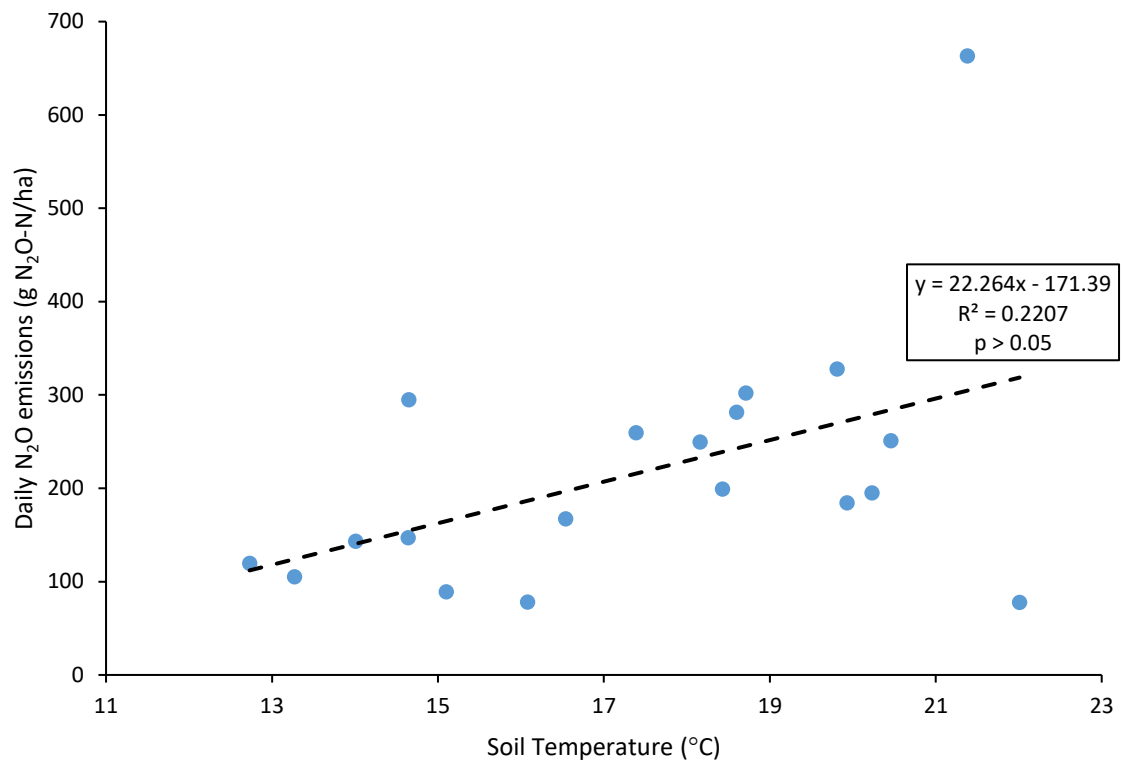


**Figure 4.28: The relationship between combined logarithmic transformed daily N<sub>2</sub>O emissions and water-filled pore space (WFPS).**

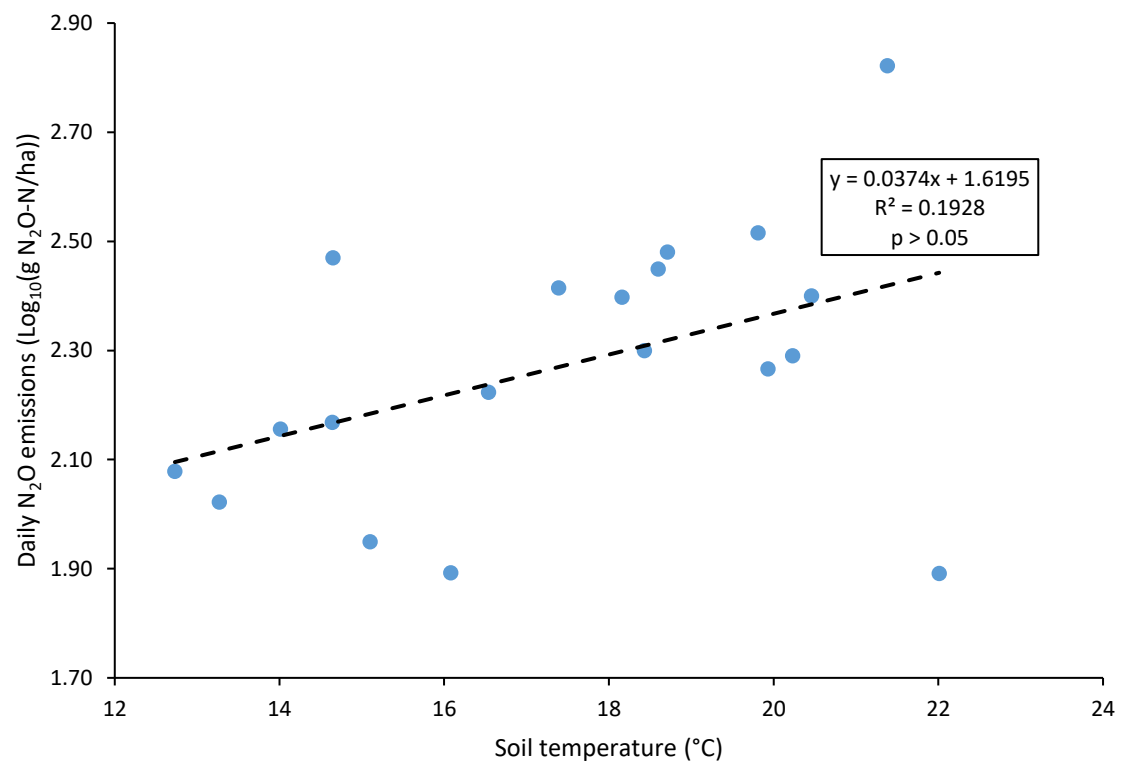
#### 4.1.2.5 Relationship between N<sub>2</sub>O emissions and Soil temperature

Under the spray irrigation with urine treatment, N<sub>2</sub>O emissions increased with soil temperature. The standout point on the graph (Fig 4.29) showed that 663.50 g N<sub>2</sub>O-N/ha of N<sub>2</sub>O was emitted at 21°C. This might be a result of the high temperature and spray irrigation applied two days before. When the N<sub>2</sub>O data were log<sub>10</sub>-transformed, the relationship between soil temperature and N<sub>2</sub>O emissions was not improved (Fig 4.30).



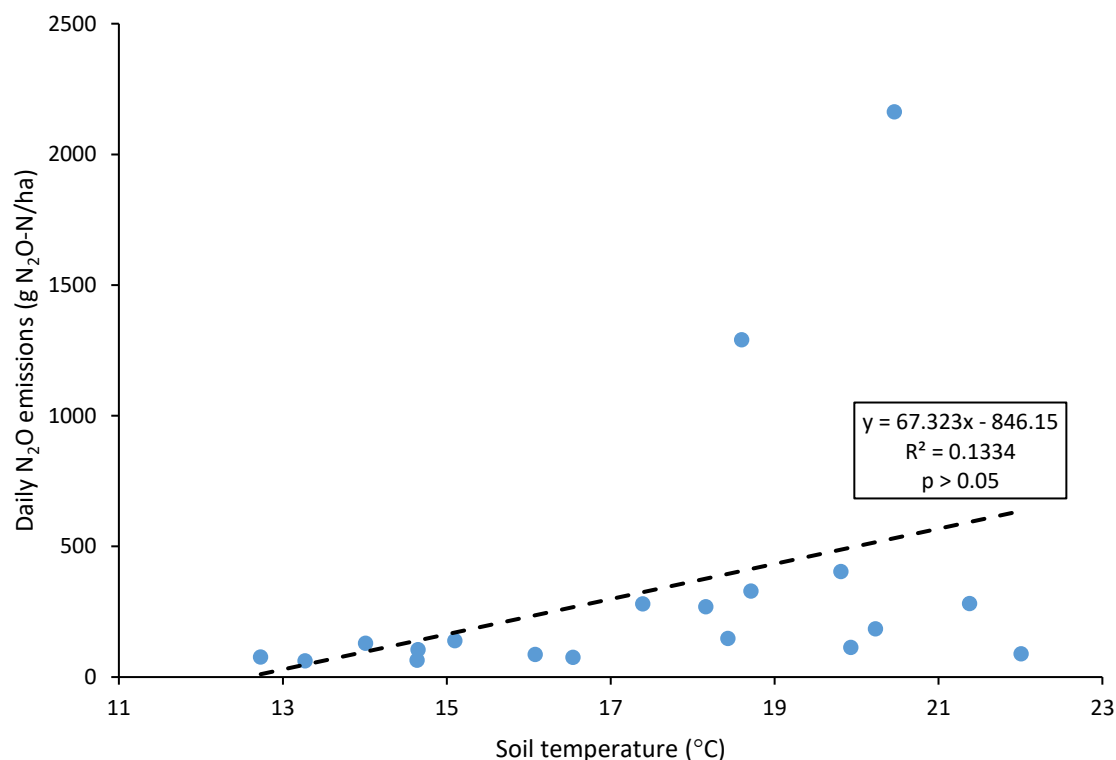


**Figure 4.29: The relationship between daily  $\text{N}_2\text{O}$  emissions and soil temperature under spray irrigation with urine treatment.**

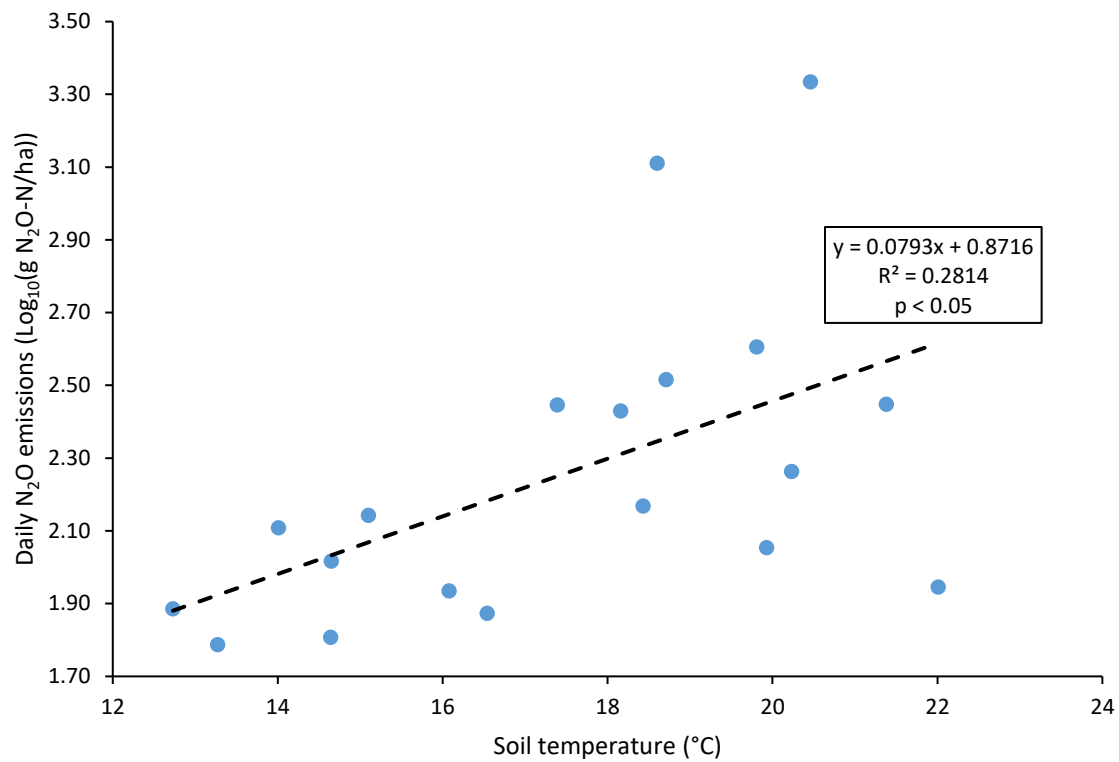


**Figure 4.30: The relationship between logarithmic transformed daily  $\text{N}_2\text{O}$  emissions and soil temperature under spray irrigation with urine treatment.**

Under the roto-rainer irrigation with urine treatment, soil temperature also influenced N<sub>2</sub>O emissions and there was a weak positive relationship (as shown in Fig 4.31). There were two standout points on the graph (Fig 4.31) where N<sub>2</sub>O emission was 1291 g N<sub>2</sub>O-N/ha when the temperature was 19°C and 2162 g N<sub>2</sub>O-N/ha when the temperature was 20°C. These might be a result of the high temperatures and roto-rainer irrigation applied on the same day and a day before respectively. When the N<sub>2</sub>O data were log<sub>10</sub>-transformed, there was a stronger positive relationship between soil temperature and N<sub>2</sub>O emissions (Fig 4.32).

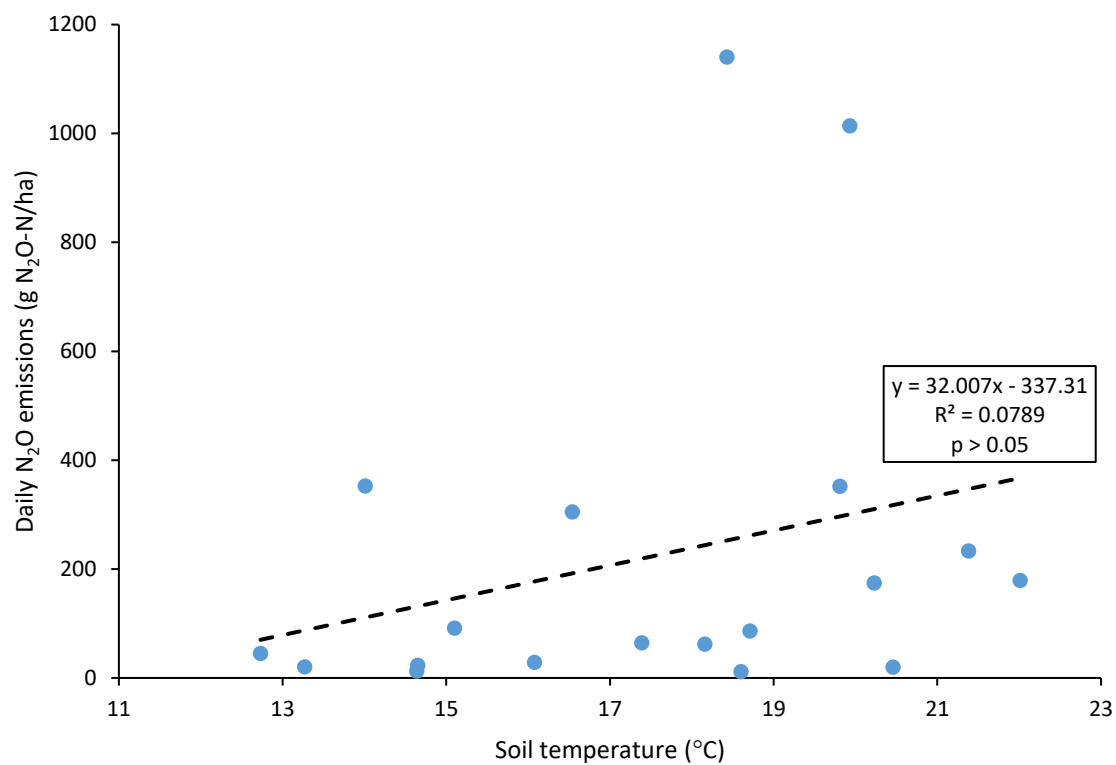


**Figure 4.31: The relationship between daily N<sub>2</sub>O emissions and soil temperature under roto-rainer irrigation with urine treatment.**

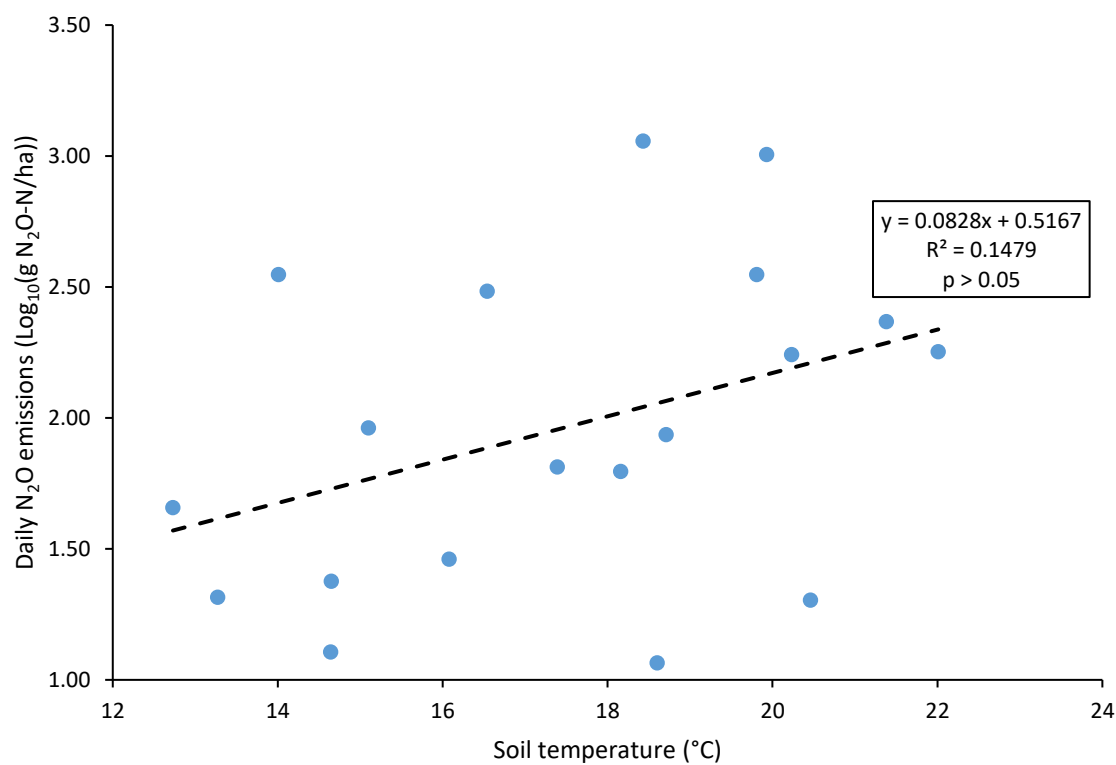


**Figure 4.32: The relationship between logarithmic transformed daily N<sub>2</sub>O emissions and soil temperature under roto-rainer irrigation with urine treatment.**

Under flood irrigation with urine treatment the results showed that soil temperature also influenced the N<sub>2</sub>O emissions. That is, N<sub>2</sub>O emissions increased with temperature (Fig 4.33). There were also two standout points which showed N<sub>2</sub>O emission was 1140 g N<sub>2</sub>O-N/ha when the temperature was 18°C and 1014 g N<sub>2</sub>O-N/ha when the temperature was 20°C. These might be a result of the high temperatures and flood irrigation applied 4 days and 3 days before respectively. When the N<sub>2</sub>O data were log<sub>10</sub>-transformed, there was a stronger positive relationship between soil temperature and N<sub>2</sub>O emissions (Fig 4.34).

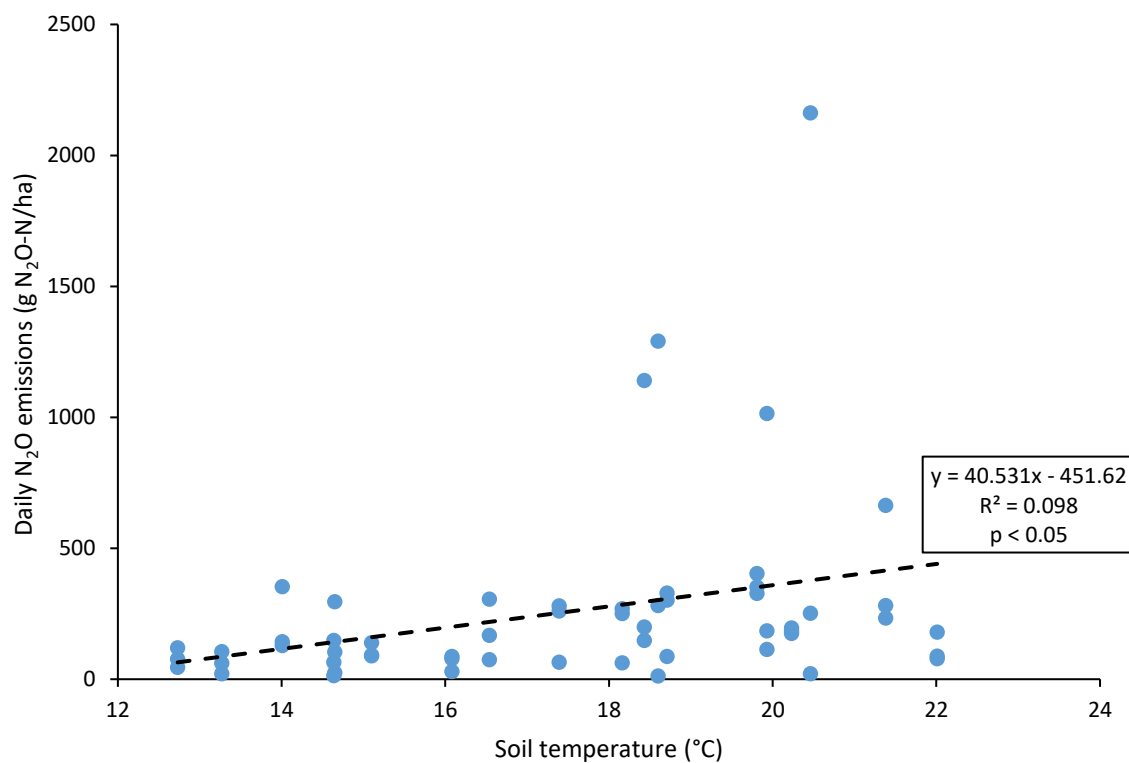


**Figure 4.33: The relationship between daily  $\text{N}_2\text{O}$  emissions and soil temperature under flood irrigation with urine treatment.**

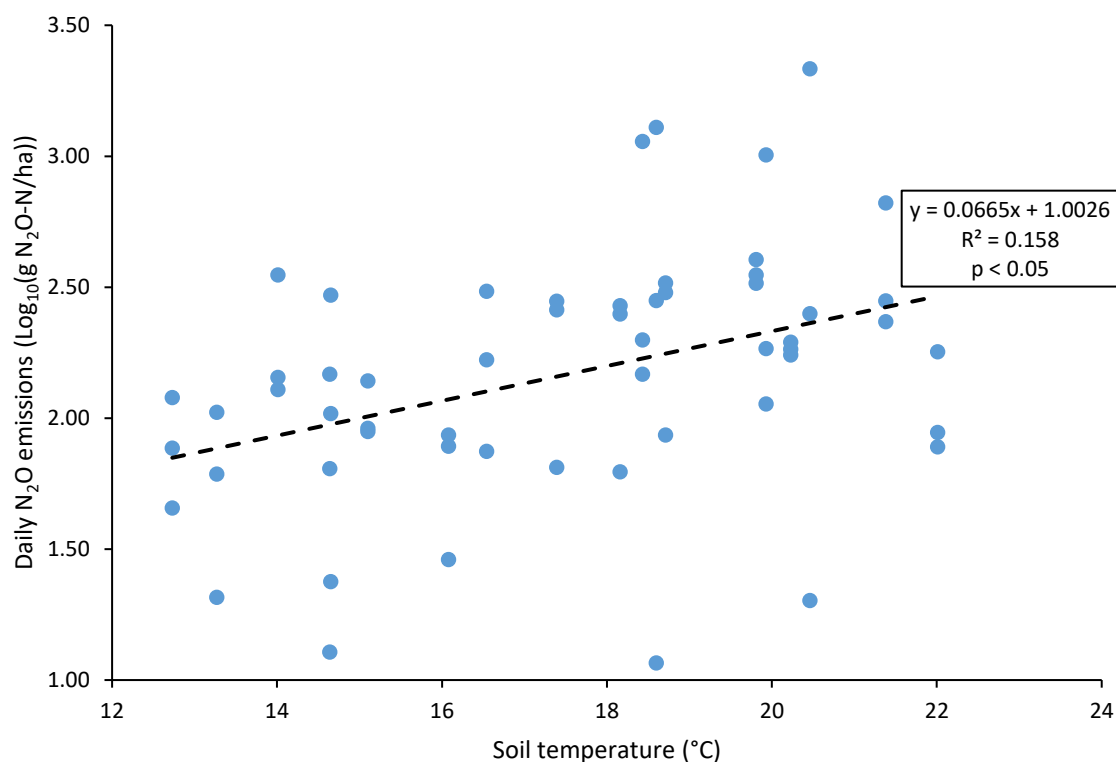


**Figure 4.34: The relationship between logarithmic transformed daily  $\text{N}_2\text{O}$  emissions and soil temperature under flood irrigation with urine treatment.**

Figures 4.35 and 4.36 showed the relationships between combined daily N<sub>2</sub>O emissions and soil temperature before log<sub>10</sub>-transformation and after log<sub>10</sub>-transformation of the data. Figure 4.35 showed a weak positive relationship between soil temperature and N<sub>2</sub>O emissions. After the data was log<sub>10</sub>-transformed, there was a stronger (but still weak) positive relationship between soil temperature and N<sub>2</sub>O emissions (Fig 4.36).



**Figure 4.35: The relationship between combined daily N<sub>2</sub>O emissions and soil temperature.**

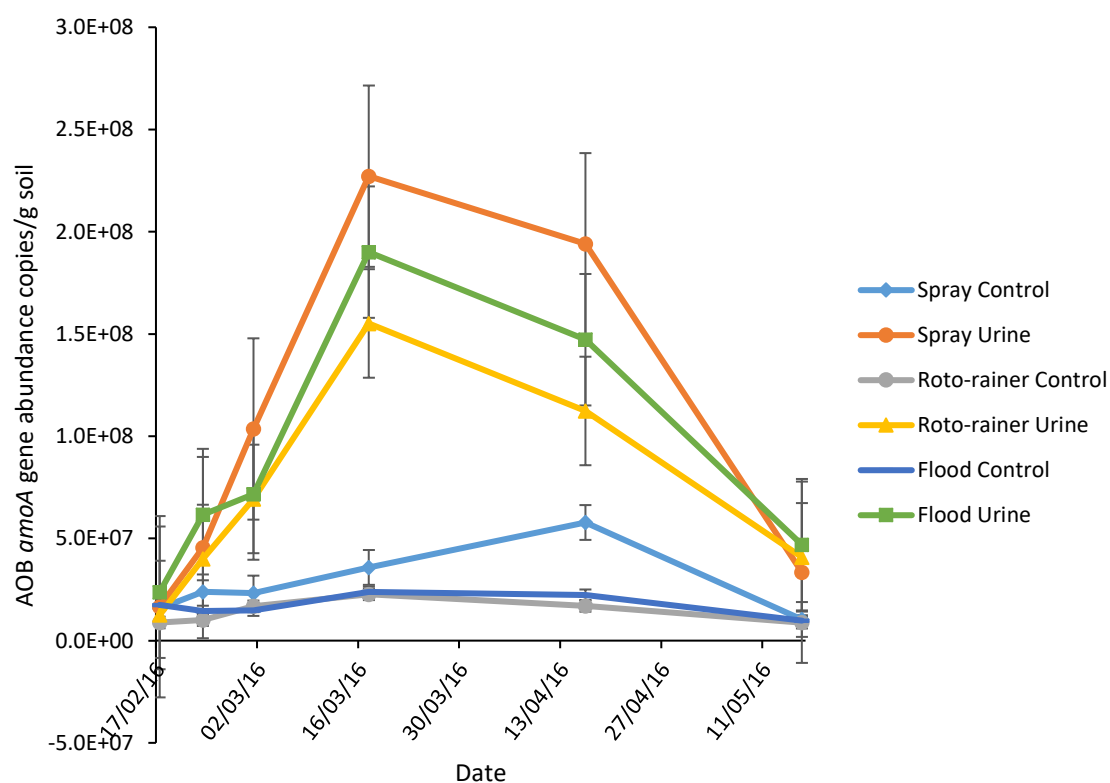


**Figure 4.36: The relationship between combined logarithmic transformed daily N<sub>2</sub>O emissions and soil temperature.**

### **4.1.3 Ammonia oxidising community abundance**

#### **4.1.3.1 Ammonia oxidising bacteria (AOB)**

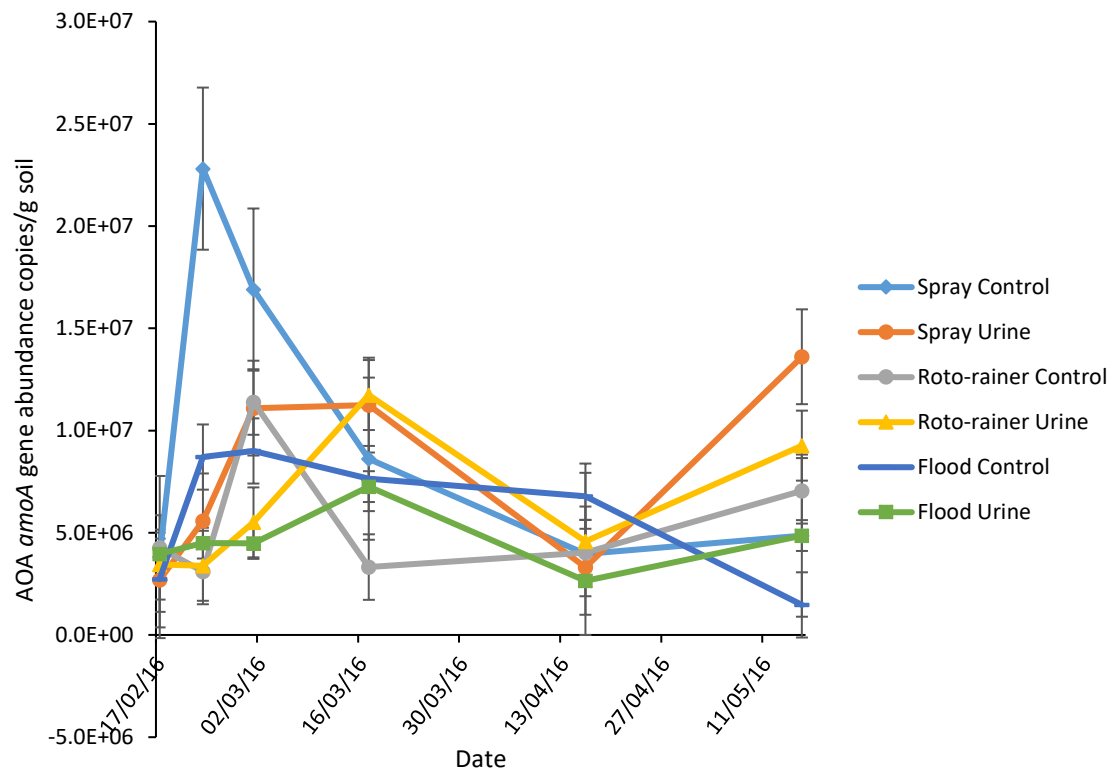
For the control treatments the irrigation system had little effect on AOB abundance. For the urine treatment under the three irrigation systems, there was an increase in AOB abundance compared to the controls. However, within the irrigation plus urine treatments, there was no significant difference between the irrigation systems except on the 17<sup>th</sup> of March where there was a significant difference between the roto-rainer urine and spray urine treatments ( $p < 0.05$ ).



**Figure 4.37: Average AOB *amoA* gene abundance from the three irrigation systems with and without urine. The error bars indicate the standard error of mean (SEM).**

#### 4.1.3.2 Ammonia oxidising archaea (AOA)

There were no significant differences in AOA abundance between the urine treatments and the controls. There was one initial peak AOA abundance in the spray control treatment but all the other values were not significantly different.



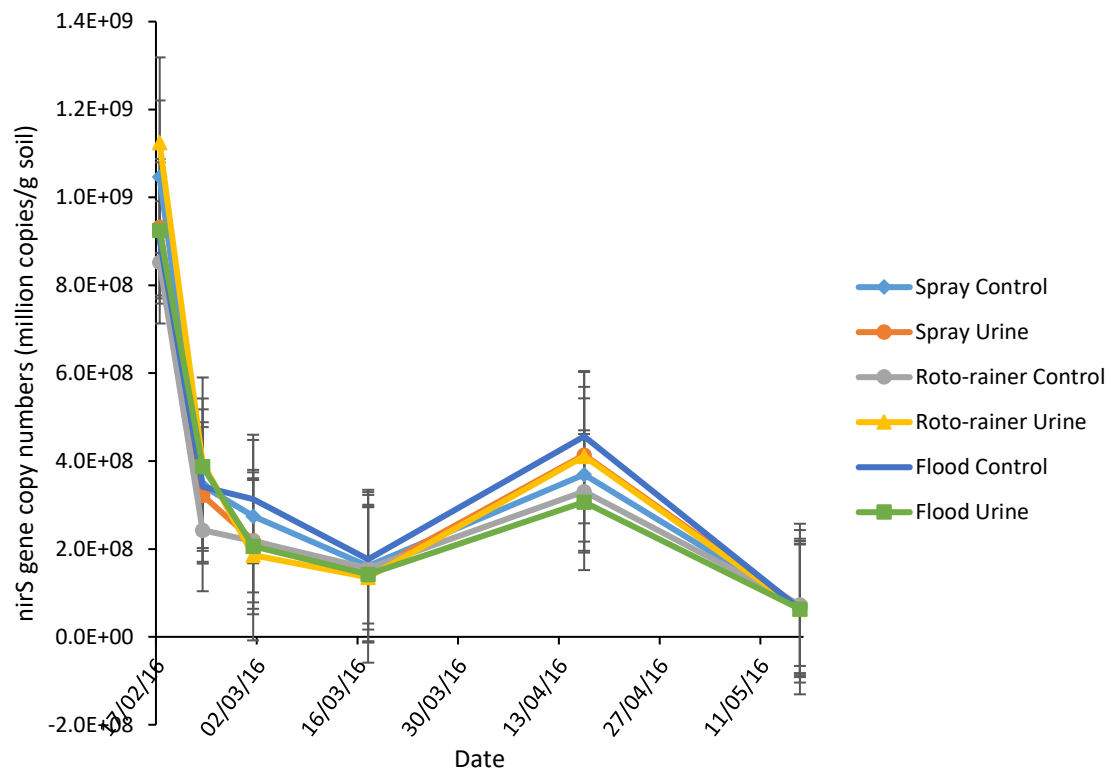
**Figure 4.38: Average AOA *amoA* gene abundance from the three irrigation systems with and without urine. The error bars indicate the standard error of mean (SEM).**

#### 4.1.4 Denitrifiers

##### 4.1.4.1 *nirS* gene abundance

There was no treatment effect on the *nirS* abundance and the irrigation systems did not have an effect on the *nirS* abundance. There was decrease in the *nirS* abundance and then there was a small peak on the 16<sup>th</sup> of April for all treatments (Fig 4.39). After the small peak, there was a decrease in *nirS* abundance in all treatments under all three irrigation systems.

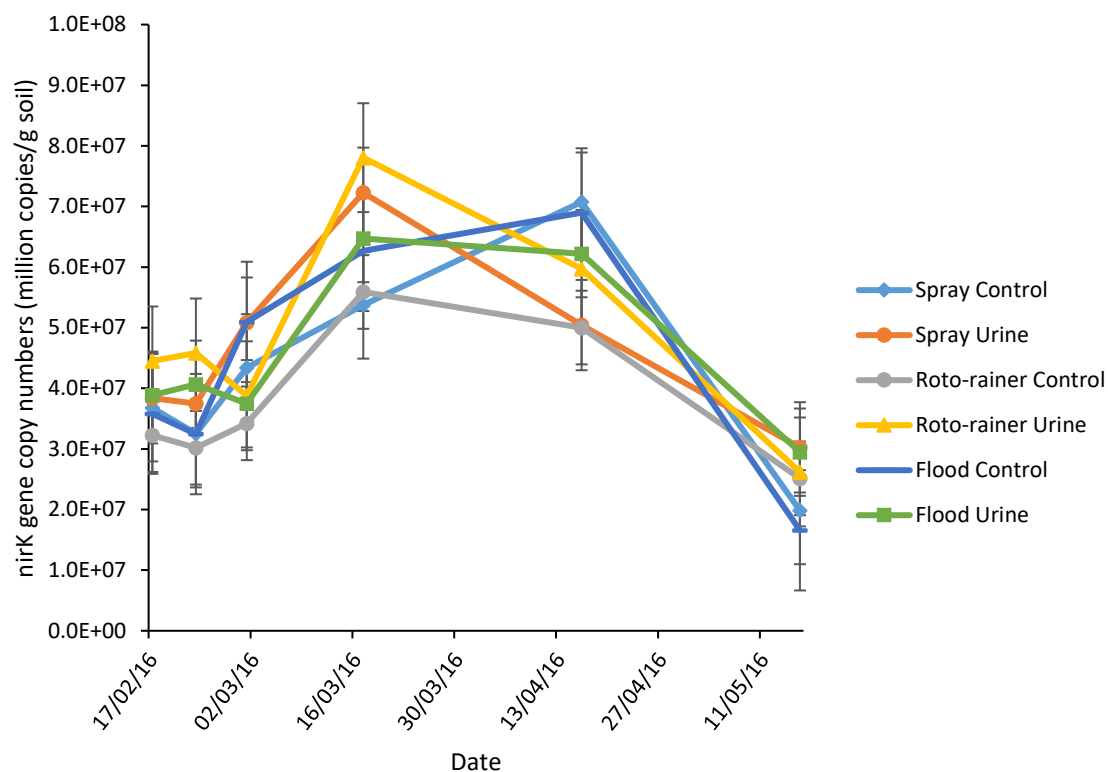




**Figure 4.39: *nirS* gene abundance from the three irrigation systems with and without urine.**  
The error bars indicate the standard error of mean (SEM).

#### 4.1.4.2 *nirK* gene abundance

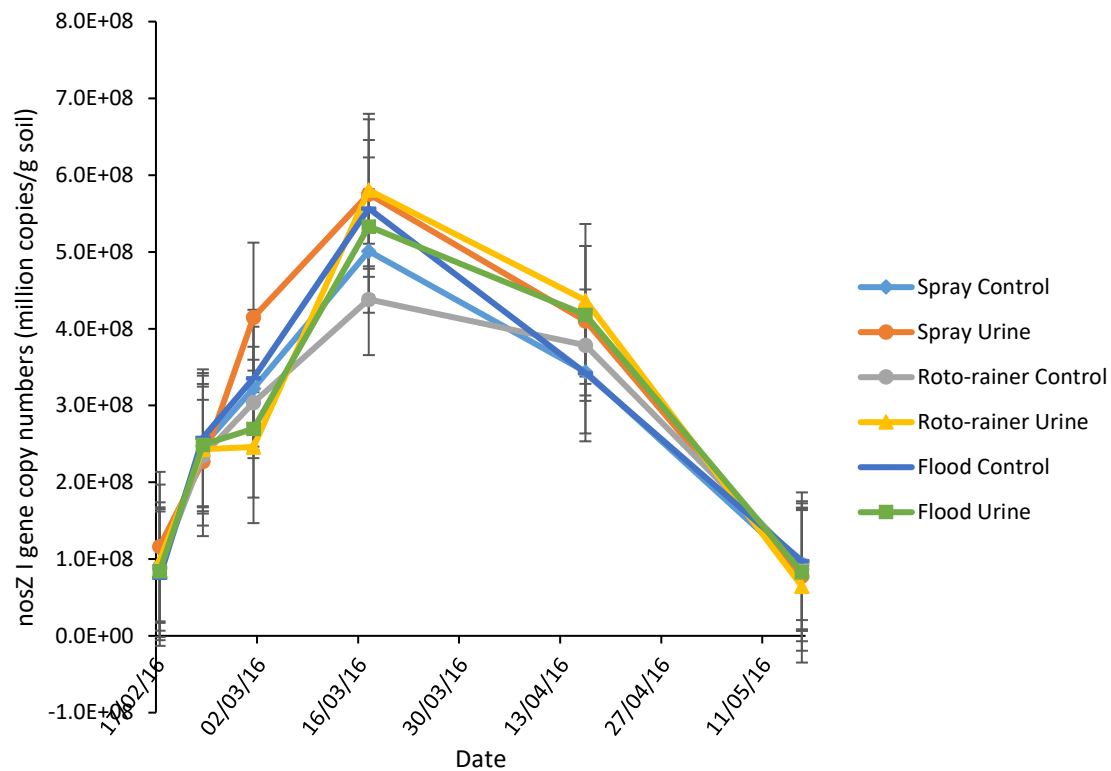
There was no treatment effect on the *nirK* abundance and the irrigation systems did not have an effect on the *nirK* abundance. There was an increase in the *nirK* abundance for all the treatments and then a decrease towards the end of the experiment (Fig 4.40).



**Figure 4.40: *nirK* gene abundance from the three irrigation systems with and without urine.**  
The error bars indicate the standard error of mean (SEM).

#### 4.1.4.3 *nosZ I* gene abundance

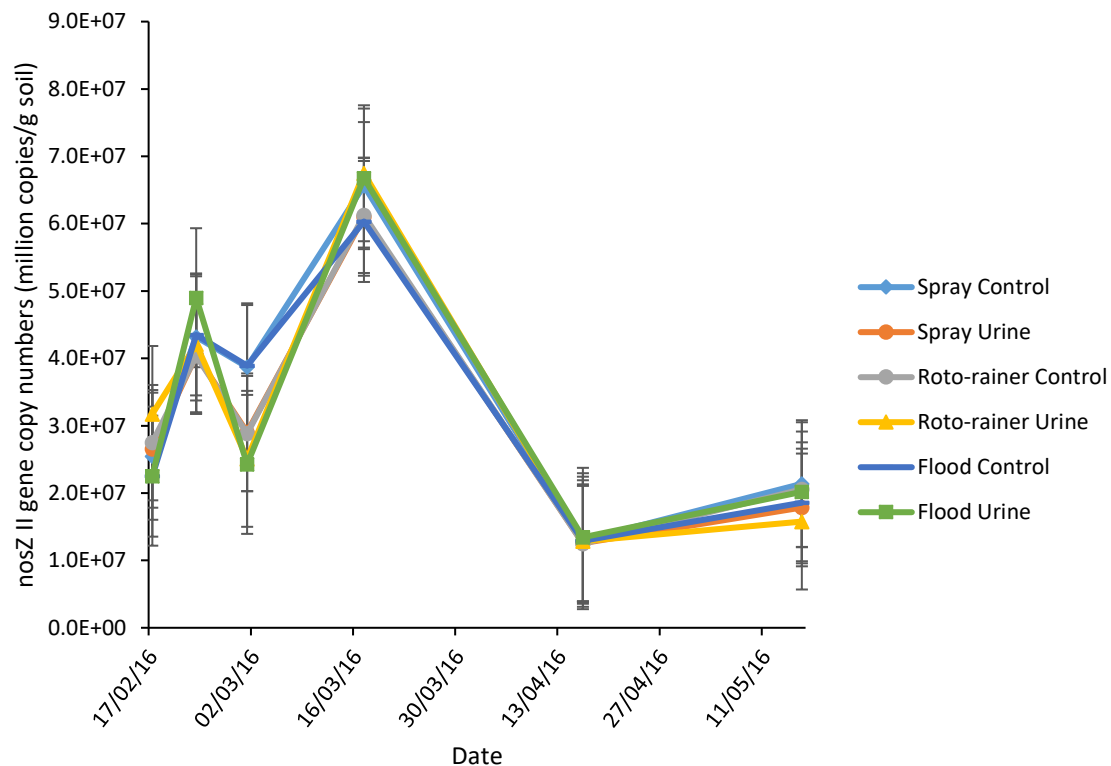
There was no treatment effect on the *nosZ I* abundance and the irrigation systems did not have an effect on the *nosZ I* abundance. There was a peak in the *nosZ I* abundance in all of the treatments on the 17<sup>th</sup> of March. After the peak, the *nosZ I* abundance decreased in all of the treatments (Fig 4.41).



**Figure 4.41: *nosZ I* gene abundance from the three irrigation systems with and without urine. The error bars indicate the standard error of mean (SEM).**

#### 4.1.4.4 *nosZ II* gene abundance

There was no treatment effect on the *nosZ II* abundance and the irrigation systems did not have an effect on the *nosZ II* abundance. There was a small peak in the *nosZ II* abundance in all of the treatments on the 23<sup>rd</sup> of February and then it decreased. On the 17<sup>th</sup> of March *nosZ II* gene abundance peaked again and then decreased. There was a slight increase in the *nosZ II* abundance in all of the treatments towards the end of the experiment (Fig 4.42).

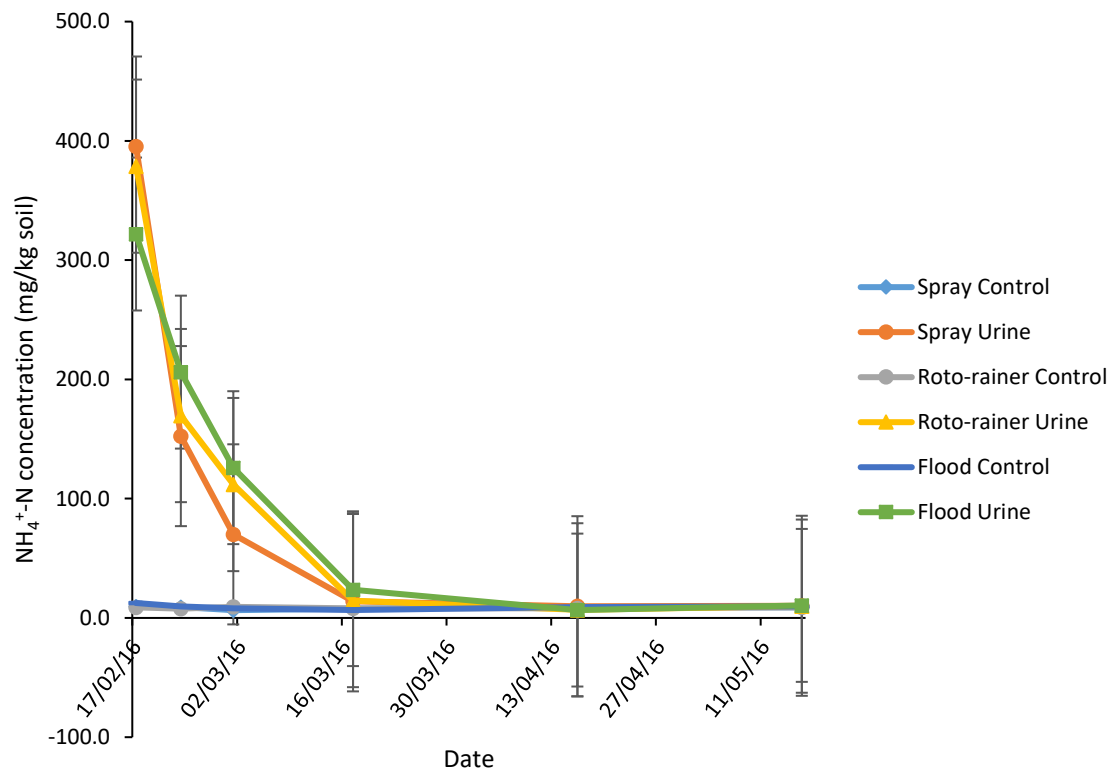


**Figure 4.42: *nosZ II* gene abundance from the three irrigation systems with and without urine. The error bars indicate the standard error of mean (SEM).**

#### 4.1.5 Soil mineral nitrogen

##### 4.1.5.1 Soil $\text{NH}_4^+$ concentrations

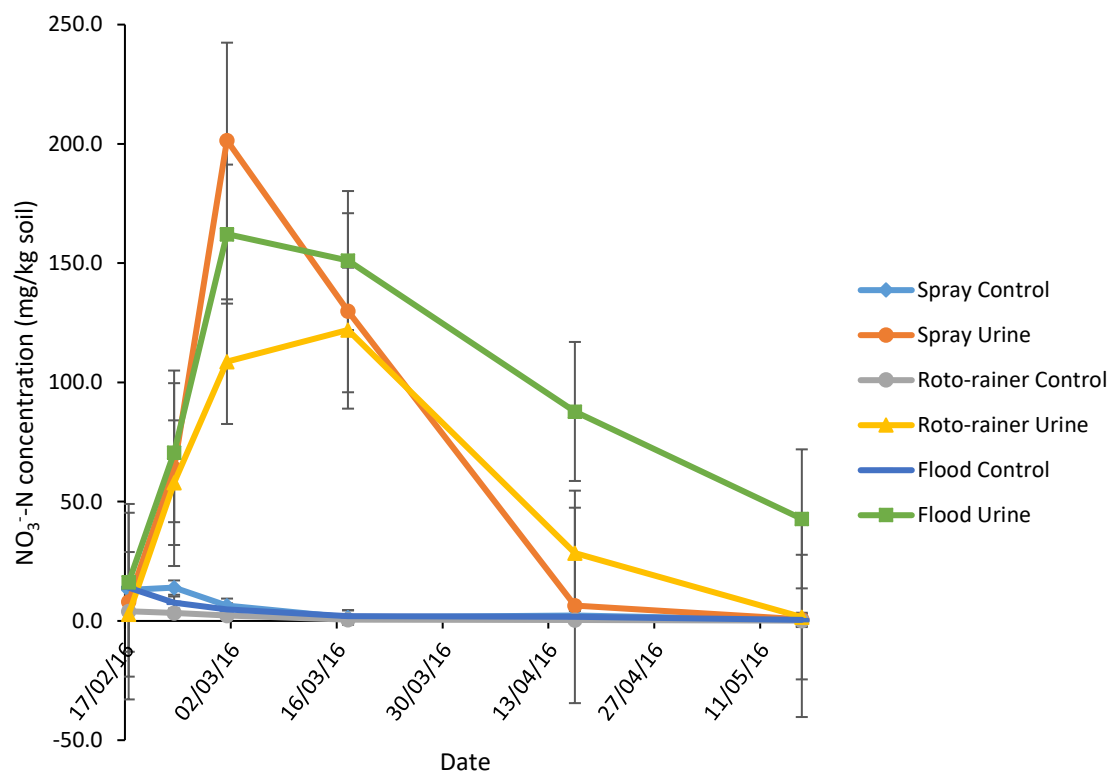
For the control treatments, there was no difference in  $\text{NH}_4^+$  concentrations under the three irrigation systems. Following the urine treatment, the  $\text{NH}_4^+$  concentrations were higher than the controls and then declined over the period from application until reaching values similar to the controls on 16 March 2016. There were no significant differences between irrigation treatments on any individual days (Fig 4.43).



**Figure 4.43: Soil ammonium concentration following dairy cow urine application (700 kg N/ha) to the three irrigation systems. The error bars indicate the standard error of mean (SEM).**

#### 4.1.5.2 Soil $\text{NO}_3^-$ concentrations

In the control treatments, there was no difference in the  $\text{NO}_3^-$  concentrations under the three irrigation systems (Fig 4.44). For the urine treatments, the  $\text{NO}_3^-$  concentrations increased under each of the three irrigation systems until they reached a peak during March and then decreased. Spray irrigation (with urine) reached the highest peak of 201 mg  $\text{NO}_3^-$  - N/kg soil on the 1<sup>st</sup> of March and then decreased (Fig 4.44).



**Figure 4.44: Soil nitrate concentration following dairy cow urine application (700 kg N/ha) to the three irrigation systems. The error bars indicate the standard error of mean (SEM).**

## Chapter 5

### Discussion and conclusions

#### 5.1 Discussion

##### 5.1.1 N<sub>2</sub>O emissions and irrigation systems

The daily N<sub>2</sub>O emissions from urine treated soil under the three irrigation systems increased when there was rainfall or irrigation applied. The rainfall or irrigation increased the soil moisture content (Figs 4.6, 4.7 & 4.8) which would have caused an increase in N<sub>2</sub>O flux (Figs 4.9, 4.10 & 4.11). These results are in agreement with those obtained by Di & Cameron (2002); Di & Cameron (2003); and Di *et al.*, (2014), where N<sub>2</sub>O emissions peaked after urine application and with increased soil moisture content. When there was no rainfall or irrigation applied (in between the peaks), N<sub>2</sub>O emissions decreased. This was a result from the reduction of soil moisture content and thus increased aeration status of the soil. Soil water content is known to be an important controlling factor for denitrification (de Klein and van Logtestijn, 1994). Following irrigation or rainfall, soil water contents increased and this caused the soil to become anaerobic, which led to higher denitrification rates and increased N<sub>2</sub>O emissions (Smith and Arah, 1990; Di *et al.*, 2014). However, if the soil becomes completely anaerobic, complete denitrification can occur, which can lead to the conversion of N<sub>2</sub>O to N<sub>2</sub> (Smith *et al.*, 1998).

In the later stages of the experiment, there was a gradual decline in N<sub>2</sub>O-N flux reaching background levels despite constant rainfall and irrigation. Under the spray irrigation system N<sub>2</sub>O-N flux did not reach background levels until the end of May (Fig 4.9). However, under the roto-rainer and flood irrigation systems, N<sub>2</sub>O-N flux gradually declined close to background levels. Under these two irrigation systems there were no N<sub>2</sub>O peaks after 11<sup>th</sup> of April (Fig 4.10 & 4.11) even though irrigation for both systems continued. This was due to the low concentrations of NO<sub>3</sub><sup>-</sup> remaining in the soil during this later phase (Fig 4.44). N<sub>2</sub>O emissions as a rule increase under irrigation when there is adequate availability of soil mineral nitrogen (Trost *et al.*, 2013) and in this experiment the NO<sub>3</sub><sup>-</sup> concentration became too low after 11<sup>th</sup> of April for any N<sub>2</sub>O emissions to occur.

Total N<sub>2</sub>O emissions increased significantly after urine application compared to the non-urine (control) treatments (Fig 4.12). These results are in agreement with de Klein *et al.*, (2003); Di &

Cameron (2006); Di *et al.*, (2007); Di *et al.*, (2010b); Di *et al.*, (2014) and Treweek *et al.*'s (2016), findings which showed total N<sub>2</sub>O emissions increased with increased mineral nitrogen content in urine-treated soils.

The N<sub>2</sub>O emission factors (N<sub>2</sub>O-N emitted as a percentage of urine-N applied) under the three irrigation systems with urine treatment (spray, roto-rainer and flood) were 2%, 3% and 2% respectively. The emission factor that New Zealand adopted is 1% of the excreta N deposited during grazing (Sherlock *et al.*, 1997). Under the three irrigation systems, the emission factors were higher than New Zealand's default emission factor of 1% (de Klein *et al.*, 2003). Comparing these results with the IPCC's default emission factor of 2% (de Klein, 2004), spray and flood irrigation systems have the same emission factor (2%) but roto-rainer irrigation system has a higher emission factor (3%). The emission factors reported in this study were within the range of values presented in the review by Cameron *et al.* (2013), including similar studies on sandy loam soil in Canterbury and Southland (Di and Cameron, 2006; Di *et al.*, 2007; Di *et al.*, 2010b).

Contrary to expectations, this study did not find a significant difference between the effect of the three irrigation systems on N<sub>2</sub>O emissions. The hypothesis that the method of irrigation will affect the N<sub>2</sub>O emissions should therefore be rejected.

### **5.1.2 Soil water content (SWC)**

The results of this study showed that there was a weak negative relationship between SWC and N<sub>2</sub>O emissions under spray irrigation with urine treatment. For the roto-rainer and flood irrigation with urine treatment, there was a weak positive relationship between SWC and N<sub>2</sub>O emissions; however it is important to note that most of the N<sub>2</sub>O emissions took place at SWCs below field capacity (Fig 4.16 and 4.18). These N<sub>2</sub>O emissions taking place below the field capacity may be a result of the oxidative nitrification process dominating (Horváth *et al.*, 2010). These results are not in agreement with those obtained by Davidson, (1991); Dobbie *et al.*, (1999); Hedley *et al.*, (2002); Saggar *et al.*, (2004a); and Di *et al.*, (2014), where N<sub>2</sub>O emissions increased with increased SWC above field capacity. The difference may be because this study examined the effect of different irrigation systems on N<sub>2</sub>O emissions which occurred mostly below field capacity, whereas the other studies examined soil moisture vs N<sub>2</sub>O relationships at higher soil moisture contents (i.e. above field capacity) which occur during winter months.



### 5.1.3 Soil water-filled pore space (WFPS)

In this study there was a weak negative relationship between WFPS and N<sub>2</sub>O emissions under spray irrigation with urine treatment (Fig 4.21 & 4.22). For the roto-rainer and flood irrigation with urine treatment, there was a weak positive relationship between WFPS and N<sub>2</sub>O emissions (Fig 4.23 & Fig 4.25) and most of the N<sub>2</sub>O emissions took place below the WFPS at field capacity. Again these N<sub>2</sub>O emissions taking place below the field capacity may be a result of the oxidative nitrification process dominating. The relationship between the daily N<sub>2</sub>O emissions (logarithmic transformed) and WFPS for roto-rainer and flood irrigation was shown in Fig 4.24 and Fig 4.26. These results are not in agreement with previous studies (e.g., Ruz-Jerez *et al.*, 1994; Luo *et al.*, 1999b, 2000, 2008; Anger *et al.*, 2003; de Klein *et al.*, 2003), which showed that N<sub>2</sub>O emissions were high when the soil WFPS was above field capacity.

Luo *et al.*, (2008) showed that N<sub>2</sub>O emissions were strongly influenced by soil WFPS; the emissions were high when soil WFPS was above field capacity during winter and spring and low when soil WFPS was below field capacity during summer and autumn. A likely explanation in the difference between this current study and the previous studies is that this study examined the effect of different irrigation systems on N<sub>2</sub>O emissions which occurred mostly below field capacity, whereas the other studies examined WFPS vs N<sub>2</sub>O relationships at higher soil water-filled pore space (i.e. above field capacity) and often during winter months.

### 5.1.4 Soil temperature

Soil temperature influenced N<sub>2</sub>O emissions under the spray, roto-rainer and flood irrigation systems with urine treatment (Figs 4.29, 4.31 & 4.33). N<sub>2</sub>O emissions increased when soil temperatures increased from 12°C to 21°C under spray irrigation, 12°C to 20°C under roto-rainer irrigation and 12°C to 18°C under flood irrigation. Increased N<sub>2</sub>O emissions was a result from increased nitrification and denitrification which was triggered by the increased soil temperatures. These results are in agreement with those obtained by Ryden, (1986); de Klein & van Logtestijn, (1996); Dobbie *et al.*, (1999); and Dobbie & Smith, (2001).

### 5.1.5 Ammonia oxidising communities

In the urine treatments, the AOB *amoA* gene abundance increased compared to the control treatments. This was due to the increase in ammonium substrate available in the urine treatments. These results are in agreement with those of previous studies (Di *et al.*, 2009a, 2010a, 2014; Robinson *et al.*, 2014; and Long *et al.*, 2016).

In contrast, the AOA *amoA* gene abundance did not increase under the urine treatment under the three irrigation systems. This is also in agreement with the findings of others (e.g., Di *et al.*, 2009a, 2010a, 2014; Long *et al.*, 2016) who reported no response in AOA abundance when urine was applied to soil. Though there was a variation, AOA abundance was lower than AOB abundance in the urine treated soil under the three irrigation systems. These results are in agreement with findings of Di *et al.* (2014) which showed AOB growth was stimulated by the application of animal urine (but not AOA growth).

### 5.1.6 Soil denitrifiers

This study showed that *nirS* abundance decreased and *nirK* abundance increased under the three irrigation systems with and without urine. In contrast to this study, Di *et al.* (2014) showed that application of urine decreased the abundance of *nirS*. It was interesting in this study to find that, for the control treatment under the three irrigation systems, *nirK* abundance also increased but their copy numbers were slightly lower than the urine treatment. Studies carried out by Di *et al.*, (2014); Long *et al.*, (2016) and Treweek *et al.*, (2016a) found that *nirK* abundance increased as a result of the increase in nitrate substrate.

This study found that *nosZ* I and II gene abundance increased to reach a peak after one month and then decreased for all the treatments (control and urine) under the three irrigation systems. This showed that there was no difference between *nosZ* I and II gene abundance under the three irrigation systems with and without urine. In contrast to this study, Di *et al.*, (2014) found that *nosZ* I and II abundance significantly increased after urine application. Again the differences between soil moisture contents of these studies (i.e. below field capacity in this study vs above field capacity in other studies) may be responsible for the differences in the observations.

### 5.1.7 Soil mineral nitrogen

The concentrations of  $\text{NH}_4^+$  in the urine treatments under the three irrigation systems were high immediately after urine application and decreased over time, showing that nitrification was taking place (Fig 4.43). The AOB used this  $\text{NH}_4^+$  as substrate for growth (as described above and shown in Fig 4.37). In contrast to  $\text{NH}_4^+$  concentration,  $\text{NO}_3^-$  concentration increased soon after urine treatment under the three irrigation systems confirming that nitrification was occurring (Figure 4.44).

These results are in line with the studies carried out by Ball *et al.*, (2012); Di *et al.*, (2014); Long *et al.*, (2016); and Treweek *et al.*, (2016a). The  $\text{NO}_3^-$  concentration decreased after one month as the  $\text{NH}_4^+$  substrate was used up by the AOB, and as the  $\text{NO}_3^-$  and  $\text{NH}_4^+$  ions were taken up by plants or may have been leached.

## 5.2 Conclusions

The results showed that:

- Methods of irrigation studied (spray vs roto-rainer vs flood) had no significant effect on total N<sub>2</sub>O-N emissions. Thus the hypothesis that the method of irrigation will affect the N<sub>2</sub>O emissions should be rejected.
- Methods of irrigation affected the N<sub>2</sub>O-N emissions' temporal patterns with N<sub>2</sub>O emissions continuing throughout the full period from spray irrigation whilst N<sub>2</sub>O emissions reached background levels on the 11<sup>th</sup> of April for roto-rainer and flood irrigation.
- Irrigation systems did not significantly affect the AOB, AOA or denitrifier abundance, which helps to explain the similar total N<sub>2</sub>O-N emissions between the irrigation treatments.
- The EF<sub>3</sub> values for these irrigation treatments (2% for spray, 3% for roto-rainer, and 2% for flood) were higher than the New Zealand's specific EF<sub>3</sub> value. This is probably a result of the warm soil temperatures combined with moist soil conditions under irrigation.
- There was no significant relationship between N<sub>2</sub>O-N emissions and soil WFPS for the spray irrigation.
- The relationship between WFPS for the roto-rainer and flood irrigation treatments was stronger than the relationship for spray irrigation (but was still weak due to the WFPS values mostly being less than those at field capacity soil moisture content).
- Importantly, the results showed that considerable amounts of N<sub>2</sub>O-N can be emitted from soils at moisture contents below field capacity.
- There was a positive relationship between soil temperature and N<sub>2</sub>O-N emissions.

### 5.3 Future research

From the results gathered from this study, it is suggested that future research into the effect of irrigation systems (spray vs roto-rainer vs flood) on N<sub>2</sub>O emissions from urine applied to pasture soil be carried out

- for different soil types, because only one soil type (Templeton sandy loam soil/Paparua soil) was studied.
- for different pasture types (e.g. standard perennial ryegrass/white clover vs “diverse pasture”).
- for spring/early summer (e.g. October) urine application and subsequent irrigation over summer and autumn.
- for different rates of urine application.

## Appendix A

### Soil Properties

**Table 1.1: Surface (0-20 cm) soil properties of Canterbury Templeton soil used for the study (Adapted from Soil Bureau Bulletin 26, 1998; Di *et al.*, 2007; Carlton *et al.*, 2016).**

Properties	Canterbury Templeton soil
Particle size distribution (%)	
Sand	40.6
Silt	42.8
Clay	16.6
Organic C (mg C g <sup>-1</sup> )	24.0
Total N (mg N g <sup>-1</sup> )	2.0
pH (H <sub>2</sub> O)	5.8
CEC (cmol <sub>c</sub> kg <sup>-1</sup> )	24.0
Base saturation (%)	47
Field capacity water content (%) (V/V)	33

## References

- Abbasi M. K., Adams W. A. (2000). Gaseous N emission during simultaneous nitrification–denitrification associated with mineral N fertilization to a grassland soil under field conditions. *Soil Biology and Biochemistry*, **32**(8), 1251-1259.
- Anger M., Hoffmann C., Kühbauch W. (2003). Nitrous oxide emissions from artificial urine patches applied to different N-fertilized swards and estimated annual N<sub>2</sub>O emissions for differently fertilized pastures in an upland location in Germany. *Soil Use and Management*, **19**(2), 104-111.
- Aulakh M. S., Doran J. W., Mosier A. R. (1991). Field Evaluation of Four Methods for Measuring Denitrification. *Soil Science Society of America Journal*, **55**(5), 1332-1338.
- Bakken L. R., Bergaust L., Liu B., Frostegård Å. (2012). Regulation of denitrification at the cellular level: a clue to the understanding of N<sub>2</sub>O emissions from soils. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **367**(1593), 1226-1234.
- Ball B. C., Cameron K. C., Di H. J., Moore S. (2012). Effects of trampling of a wet dairy pasture soil on soil porosity and on mitigation of nitrous oxide emissions by a nitrification inhibitor, dicyandiamide. *Soil Use and Management*, **28**(2), 194-201.
- Bateman E. J., Baggs E. M. (2005). Contributions of nitrification and denitrification to N<sub>2</sub>O emissions from soils at different water-filled pore space. *Biology and Fertility of Soils*, **41**(6), 379-388.
- Bolan N. S., Saggar S., Luo J., Bhandral R., Singh J. (2004). Gaseous Emissions of Nitrogen from Grazed Pastures: Processes, Measurements and Modelling, Environmental Implications, and Mitigation *Advances in Agronomy* (Vol. Volume **84**, pp. 37-120): Academic Press.
- Bureau, New Zealand Soil. (1968). Soils of New Zealand. *New Zealand Soil Bureau Bulletin*, **26**(3), 18-125.
- Cameron K. C. (1992). Nitrogen in Soil *Encyclopedia of Earth System Science* (Vol. **3**, pp. 307-317). London, UK: Academic Press.
- Cameron K. C., Di H. J., Moir J. L. (2013). Nitrogen losses from the soil/plant system: a review. *Annals of Applied Biology*, **162**(2), 145-173. doi:10.1111/aab.12014
- Cameron K. C., Smith N. P., McLay C., Fraser P. M., McPherson R., Harrison D., Harbottle P. (1992). Lysimeters without edge flow: an improved design and sampling procedure. *Soil Science Society of America Journal*, **56**(5), 1625-1628.

- Carlton A. J., Cameron K. C., Edwards G. R., Di H J., Clough T. J. (2016). The effect of optimum vs. deficit irrigation on plant nitrogen uptake and nitrate leaching loss from soil. In: *Integrated nutrient and water management for sustainable farming*. (Eds L.D. Currie and R. Singh). <http://flrc.massey.ac.nz/publications.html>. Occasional Report No. 29. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand.
- Choudhary M. A., Akramkhanov A., Saggar S. (2002). Nitrous oxide emissions from a New Zealand cropped soil: tillage effects, spatial and seasonal variability. *Agriculture, Ecosystems & Environment*, **93**(1–3), 33-43.
- Dai Y., Di H. J., Cameron K. C., He J.-Z. (2013). Effects of nitrogen application rate and a nitrification inhibitor dicyandiamide on ammonia oxidizers and N<sub>2</sub>O emissions in a grazed pasture soil. *Science of The Total Environment*, **465**, 125-135.
- Davidson E. A. (1991). Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems. In: Rogers J. E. and Whitman W. B. (eds), *Microbial production and consumption of greenhouse gases: methane, nitrogen oxides and halomethanes*. Am. Soc. Microbiol., Washington, DC, USA, pp. 219-235.
- Davidson E. A. (1992). Sources of Nitric Oxide and Nitrous Oxide following Wetting of Dry Soil. *Soil Science Society of America Journal*, **56**(1), 95-102.
- de Klein C. A. M. (2004). *A review of the N<sub>2</sub>O emissions factor for excreta deposited by grazing animals (EF<sub>3PRP</sub>)*. In: *Paper Prepared as Part of the 2006 Revised Guidelines for Greenhouse Gas Inventories of the International Panel on Climate Change*.
- de Klein C. A. M., Van Logtestijn R. S. P. (1996). Denitrification in grassland soils in The Netherlands in relation to irrigation, N-application rate, soil water content and soil temperature. *Soil Biology and Biochemistry*, **28**(2), 231-237.
- de Klein C. A. M., Barton L., Sherlock R. R., Li Z., Littlejohn R. P. (2003). Estimating a nitrous oxide emission factor for animal urine from some New Zealand pastoral soils. *Soil Research*, **41**(3), 381-399.
- de Klein C. A. M., McTaggart P., Smith K. A., Stevens R. J., Harrison R., Laughlin R. J. (1999). Measurement of nitrous oxide emissions from grassland soil using photo-acoustic infra-red spectroscopy, long-path infra-red spectroscopy, gas chromatography, and continuous flow isotope-ratio mass spectrometry. *Communications in Soil Science and Plant Analysis*, **30**(9-10), 1463-1477.
- de Klein C. A. M., Pinares-Patino C., Waghorn G. C. (2008). Greenhouse Gas Emissions. In R. W. McDowell (Ed.), *Environmental Impacts of Pasture-based Farming* (pp. 1-33). USA: CAB International.
- de Klein C. A. M., Shepherd M. A., van der Weerden T. J. (2014). Nitrous oxide emissions from grazed grasslands: interactions between the N cycle and climate change — a New Zealand case study. *Current Opinion in Environmental Sustainability*, **9–10**, 131-139.



- de Klein C. A. M., Sherlock R. R., Cameron K. C., van der Weerden T. J. (2001). Nitrous oxide emissions from agricultural soils in New Zealand—A review of current knowledge and directions for future research. *Journal of the Royal Society of New Zealand*, **31**(3), 543-574.
- de Klein C. A. M., van Logtestijn R. S. P. (1994). Denitrification and N<sub>2</sub>O emission from urine-affected grassland soil. *Plant and Soil*, **163**(2), 235-241.
- Delwiche C. C. (1981). The nitrogen cycle and nitrous oxide. In C. C. Delwiche (Ed.), *Denitrification, Nitrification and atmospheric nitrous oxide* (pp. 1-15). USA: John Wiley and Sons Publishing.
- Di H., Cameron K. (2002b). Nitrate leaching and pasture production from different nitrogen sources on a shallow stoney soil under flood-irrigated dairy pasture. *Soil Research*, **40**(2), 317-334.
- Di H. J., Cameron K. C. (2006). Nitrous oxide emissions from two dairy pasture soils as affected by different rates of a fine particle suspension nitrification inhibitor, dicyandiamide. *Biology and Fertility of Soils*, **42**(6), 472-480.
- Di H. J., Cameron K. C. (2008). Sources of nitrous oxide from <sup>15</sup>N-labelled animal urine and urea fertiliser with and without a nitrification inhibitor, dicyandiamide (DCD). *Soil Research*, **46**(1), 76-82.
- Di H. J., Cameron K. C., Sherlock R. R. (2007). Comparison of the effectiveness of a nitrification inhibitor, dicyandiamide, in reducing nitrous oxide emissions in four different soils under different climatic and management conditions. *Soil Use and Management*, **23**(1), 1-9.
- Di H. J., Cameron K. C. (2002). Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. *Nutrient Cycling in Agroecosystems*, **64**(3), 237-256.
- Di H. J., Cameron K. C. (2003). Mitigation of nitrous oxide emissions in spray-irrigated grazed grassland by treating the soil with dicyandiamide, a nitrification inhibitor. *Soil Use and Management*, **19**(4), 284-290.
- Di H. J., Cameron K. C., Podolyan A., Robinson A. (2014). Effect of soil moisture status and a nitrification inhibitor, dicyandiamide, on ammonia oxidizer and denitrifier growth and nitrous oxide emissions in a grassland soil. *Soil Biology and Biochemistry*, **73**, 59-68.
- Di H. J., Cameron K. C., Shen J.-P., Winefield C. S., O'Callaghan M., Bowatte S., He J.-Z. (2010a). Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. *FEMS Microbiology Ecology*, **72**(3), 386-394.
- Di H. J., Cameron K. C., Shen J. P., He J. Z., Winefield C. S. (2009a). A lysimeter study of nitrate leaching from grazed grassland as affected by a nitrification inhibitor, dicyandiamide, and relationships with ammonia oxidizing bacteria and archaea. *Soil Use and Management*, **25**(4), 454-461.

- Di H. J., Cameron K. C., Shen J. P., Winefield C. S., O'Callaghan M., Bowatte S., He J. Z. (2009b). Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. *Nature Geosci*, **2**(9), 621-624.
- Di H. J., Cameron K. C., Sherlock R. R., Shen J.-P., He J.-Z., Winefield C. S. (2010b). Nitrous oxide emissions from grazed grassland as affected by a nitrification inhibitor, dicyandiamide, and relationships with ammonia-oxidizing bacteria and archaea. *Journal of Soils and Sediments*, **10**(5), 943-954.
- Dobbie K. E., McTaggart I. P., Smith K. A. (1999). Nitrous oxide emissions from intensive agricultural systems: Variations between crops and seasons, key driving variables, and mean emission factors. *Journal of Geophysical Research: Atmospheres*, **104**(D21), 26891-26899.
- Dobbie K. E., Smith K. A. (2001). The effects of temperature, water-filled pore space and land use on N<sub>2</sub>O emissions from an imperfectly drained gleysol. *European Journal of Soil Science*, **52**(4), 667-673.
- Ferguson S. J., Richardson D. J., van Spanning R. J. M. (2007). Biochemistry and molecular biology of nitrification. In H. Bothe, S. J. Ferguson, & W. E. Newton (Eds.), *Biology of the Nitrogen Cycle* (pp. 209-222). Amsterdam, the Netherlands: Elsevier.
- Food and Agriculture Organisation of the United Nations (FAO). (1996). Rome declaration on world food security and world food summit plan of action. Rome: Food and Agriculture Organization of the United Nations.
- Food and Agriculture Organisation of the United Nations (FAO). (2002). *World agriculture: towards 2015/2030 (Summary report)*. Rome, Italy.
- Food and Agriculture Organisation of the United Nations (FAO). (2010). Statistical yearbook (2010). Retrieved 11/10/2016 <http://www.fao.org/economic/ess/ess-publications/ess-yearbook/ess-yearbook2010/yearbook2010-resources/en/>
- Francis C. A., Roberts K. J., Beman J. M., Santoro A. E., Oakley B. B. (2005). Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Sciences of the United States of America*, **102**(41), 14683-14688.
- Fraser P. M., Cameron K. C., Sherlock R. R. (1994). Lysimeter study of the fate of nitrogen in animal urine returns to irrigated pasture. *European Journal of Soil Science*, **45**(4), 439-447.
- Garcia-Montiel D. C., Steudler P. A., Piccolo M., Neill C., Melillo J., Cerri C. C. (2003). Nitrogen oxide emissions following wetting of dry soils in forest and pastures in Rondônia, Brazil. *Biogeochemistry*, **64**(3), 319-336. doi:10.1023/a:1024968802018
- Gödde M., Conrad R. (2000). Influence of soil properties on the turnover of nitric oxide and nitrous oxide by nitrification and denitrification at constant temperature and moisture. *Biology and Fertility of Soils*, **32**(2), 120-128.

- Guo X., Drury C. F., Yang X., Reynolds W. D., Fan R. (2014). The Extent of Soil Drying and Rewetting Affects Nitrous Oxide Emissions, Denitrification, and Nitrogen Mineralization. *Soil Science Society of America Journal*, **78**(1), 194-204.
- Hallin S., Lindgren P.-E. (1999). PCR detection of genes encoding nitrite reductase in denitrifying bacteria. *Applied and environmental microbiology*, **65**(4), 1652-1657.
- Haynes R. J., Williams P. H. (1993). Nutrient Cycling and Soil Fertility in the Grazed Pasture Ecosystem. In L. S. Donald (Ed.), *Advances in Agronomy* (Vol. Volume **49**, pp. 119-199): Academic Press.
- Hedley C. B., Saggar S., Dando J. (2002). Use of intact soil cores to assess the effect of soil water content on nitrous oxide emissions from a poorly drained pasture soil. In: Back to the Future, New Zealand Soil Science Society Golden Jubilee Conference, Programme and Abstracts, 25-29 November 2002. Victoria University, Wellington, New Zealand, p. 85.
- Horváth L., Grosz B., Machon A., Tuba Z., Nagy Z., Czóbel S. Z., Balogh J., Péli E., Fóti S. Z., Weidinger T., Pintér K., Führer E. (2010). Estimation of nitrous oxide emission from Hungarian semi-arid sandy and loess grasslands; effect of soil parameters, grazing, irrigation and use of fertilizer. *Agriculture, Ecosystems & Environment*, **139**(1-2), 255-263.
- Huijing H., Shihong Y., Fangtong W., Dan L., Junzeng X. (2016). Controlled irrigation mitigates the annual integrative global warming potential of methane and nitrous oxide from the rice–winter wheat rotation systems in Southeast China. *Ecological Engineering*, **86**, 239-246.
- Hutchinson G. L., Mosier A. R. (1981). Improved Soil Cover Method for Field Measurement of Nitrous Oxide Fluxes<sup>1</sup>. *Soil Science Society of America Journal*, **45**(2), 311-316.
- Intergovernmental Panel on Climate Change (IPCC). (2007). *Climate change 2007: the physical science basis, contribution of working group I to the fourth assessment report of the intergovernmental Panel on climate change: frequently asked questions*. . Cambridge, United Kingdom.: Intergovernmental Panel on Climate Change (IPCC)
- Intergovernmental Panel on Climate Change (IPCC). (2014a). *Climate change 2013: the physical science basis: Working Group I contribution to the Fifth assessment report of the IPCC*: Cambridge University Press.
- Intergovernmental Panel on Climate Change (IPCC). (2014b). *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Retrieved from IPCC, Geneva, Switzerland:
- Jamali H., Quayle W., Scheer C., Rowlings D., Baldock J. (2016). Effect of soil texture and wheat plants on N<sub>2</sub>O fluxes: A lysimeter study. *Agricultural and Forest Meteorology*, **223**, 17-29.

- Jones C. M., Graf D. R. H., Bru D., Philippot L., Hallin, S. (2013). The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. *ISME J*, **7**(2), 417-426.
- Jones C. M., Spor A., Brennan F. P., Breuil M.-C., Bru D., Lemanceau P., Griffiths B., Hallin S., Philippot, L. (2014). Recently identified microbial guild mediates soil N<sub>2</sub>O sink capacity. *Nature Clim. Change*, **4**(9), 801-805.
- Kallenbach C. M., Rolston D. E., Horwath W. R. (2010). Cover cropping affects soil N<sub>2</sub>O and CO<sub>2</sub> emissions differently depending on type of irrigation. *Agriculture, Ecosystems & Environment*, **137**(3–4), 251-260.
- Kessavalou A, Doran J W, Mosier A R, Drijber R. A. (1998). Greenhouse Gas Fluxes following Tillage and Wetting in a Wheat-Fallow Cropping System. *Journal of Environmental Quality*, **27**(5), 1105-1116.
- Kim D.-G., Mishurov M., Kiely G. (2010). Effect of increased N use and dry periods on N<sub>2</sub>O emission from a fertilized grassland. *Nutrient Cycling in Agroecosystems*, **88**(3), 397-410.
- Kim D. G., Isenhardt T. M., Parkin T. B., Schultz R. C., Loynachan T. E., Raich J. W. (2009). Nitrous oxide emissions from riparian forest buffers, warm-season and cool-season grass filters, and crop fields. *Biogeosciences Discuss.*, 2009, 607-650.
- Klemetsson L., Simkins S., Svensson B. H., Johnsson H., Rosswall T. (1991). Soil denitrification in three cropping systems characterized by differences in nitrogen and carbon supply. *Plant and Soil*, **138**(2), 273-286.
- Liu C., Wang K., Meng S., Zheng X., Zhou Z., Han S., Chen D., Yang Z. (2011). Effects of irrigation, fertilization and crop straw management on nitrous oxide and nitric oxide emissions from a wheat–maize rotation field in northern China. *Agriculture, Ecosystems & Environment*, **140**(1–2), 226-233.
- Long X.-E., Shen J.-P., Wang J.-T., Zhang L.-M., Di H., He J.-Z. (2016). Contrasting response of two grassland soils to N addition and moisture levels: N<sub>2</sub>O emission and functional gene abundance. *Journal of Soils and Sediments*, 1-9.
- Luo J., de Klein C. A. M., Ledgard S. F., Saggar S. (2010a). Management options to reduce nitrous oxide emissions from intensively grazed pastures: A review. *Agriculture, Ecosystems & Environment*, **136**(3–4), 282-291.
- Luo J., Ledgard S. F., Lindsey S. B. (2008). A test of a winter farm management option for mitigating nitrous oxide emissions from a dairy farm. *Soil Use and Management*, **24**(2), 121-130.
- Luo J., Tillman R. W., Ball P. R. (2000). Nitrogen loss through denitrification in a soil under pasture in New Zealand. *Soil Biology and Biochemistry*, **32**(4), 497-509.
- Luo J., Tillman R. W., White R. E., Ball P. R. (1998). Variation in denitrification activity with soil depth under pasture. *Soil Biology and Biochemistry*, **30**(7), 897-903.

- Luo J., Tillman R. W., Ball. P. R. (1999b). Factors regulating denitrification in a soil under pasture. *Soil Biology and Biochemistry*, **31**(6), 913-927.
- M2 Communications. (2015). PM addresses Paris Climate Change Conference [Press release]. Retrieved from <http://go.galegroup.com/ps/i.do?id=GALE%7CA436336979&v=2.1&u=lincoln1&it=r&p=ITOF&sw=w&asid=9b716160649871d0699c4313a077b29d>
- Maag M., Vinther F. P. (1996). Nitrous oxide emission by nitrification and denitrification in different soil types and at different soil moisture contents and temperatures. *Applied Soil Ecology*, **4**(1), 5-14.
- Maharjan B., Venterea R. T., Rosen C. (2014). Fertilizer and Irrigation Management Effects on Nitrous Oxide Emissions and Nitrate Leaching. *Agronomy Journal*, **106**(2), 703-714.
- Maris S. C., Teira-Esmatges M. R., Arbonés A., Rufat J. (2015). Effect of irrigation, nitrogen application, and a nitrification inhibitor on nitrous oxide, carbon dioxide and methane emissions from an olive (*Olea europaea* L.) orchard. *Science of The Total Environment*, **538**, 966-978.
- Michotey V., Méjean V., Bonin P. (2000). Comparison of methods for quantification of cytochrome cd 1-denitrifying bacteria in environmental marine samples. *Applied and environmental microbiology*, **66**(4), 1564-1571.
- Ministry for Primary Industries. (2014). Primary Industries: Production and Trade-June Quarter. Wellington, New Zealand: Ministry for Primary Industries.
- Ministry for the Environment. (2009). New Zealand's Greenhouse Gas Inventory 1990-2007. Wellington, New Zealand: Ministry for the Environment.
- Ministry for the Environment. (2015). New Zealand's Greenhouse Gas Inventory 1990-2013. Wellington, New Zealand: Ministry for the Environment.
- Mørkved P. T., Dörsch P., Bakken L. R. (2007). The N<sub>2</sub>O product ratio of nitrification and its dependence on long-term changes in soil pH. *Soil Biology and Biochemistry*, **39**(8), 2048-2057.
- Müller C., Sherlock R. R. (2004). Nitrous oxide emissions from temperate grassland ecosystems in the Northern and Southern Hemispheres. *Global Biogeochemical Cycles*, **18**(1).
- Nobre A., Keller M., Crill P., Harriss R. (2001). Short-term nitrous oxide profile dynamics and emissions response to water, nitrogen and carbon additions in two tropical soils. *Biology and Fertility of Soils*, **34**(5), 363-373.
- Nômmik H., Larsson K. (1989). Assessment of fertilizer nitrogen accumulation in *Pinus sylvestris* trees and retention in soil by 15N recovery technique. *Scandinavian Journal of Forest Research*, **4**(1-4), 427-442.

- Nunn P. D. (2013). The end of the Pacific? Effects of sea level rise on Pacific Islands livelihoods. *Singapore Journal of Tropical Geography*, **34**(2013), 143-171.
- Owens J., Clough T. J., Laubach J., Hunt J. E., Venterea R. T., Phillips R. L. (2016). Nitrous Oxide Fluxes, Soil Oxygen, and Denitrification Potential of Urine- and Non-Urine-Treated Soil under Different Irrigation Frequencies. *Journal of Environmental Quality*, **45**(4), 1169-1177.
- Parkin T. B., Sexstone A. J., Tiedje J. M. (1985). Comparison of Field Denitrification Rates Determined by Acetylene-Based Soil Core and Nitrogen-15 Methods1. *Soil Science Society of America Journal*, **49**(1), 94-99.
- Paustian K., Lehmann J., Ogle S., Reay D., Robertson G. P., Smith P. (2016). Climate-smart soils. *Nature*, **532**(7597), 49-57.
- Prade K., Trolldenier G. (1988). Effect of wheat roots on denitrification at varying soil air-filled porosity and organic-carbon content. *Biology and Fertility of Soils*, **7**(1), 1-6.
- Prosser J. I. (2007). The ecology of nitrifying bacteria. In H. Bothe, S. J. Ferguson, & W. E. Newton (Eds.), *Biology of the Nitrogen Cycle* (pp. 223-244). Amsterdam, the Netherlands: Elsevier.
- Rafique R., Hennessy D., Kiely G. (2011). Nitrous Oxide Emission from Grazed Grassland Under Different Management Systems. *Ecosystems*, **14**(4), 563-582.
- Regaert D., Aubinet M., Moureaux C. (2015). Mitigating N<sub>2</sub>O emissions from agriculture: A review of the current knowledge on soil system modelling, environmental factors and management practices influencing emissions. *J. Soil Sci. and Environ. Manage.*, **6**(7), 178-186.
- Robinson A., Di H. J., Cameron K. C., Podolyan A., He J. (2014). The effect of soil pH and dicyandiamide (DCD) on N<sub>2</sub>O emissions and ammonia oxidiser abundance in a stimulated grazed pasture soil. *Journal of Soils and Sediments*, **14**(8), 1434-1444.
- Rotthauwe J.-H., Witzel K.-P., Liesack W. (1997). The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Applied and environmental microbiology*, **63**(12), 4704-4712.
- Ruz-Jerez B. E., White R. E., Roger., B. P. (1994). Long-term measurement of denitrification in three contrasting pastures grazed by sheep. *Soil Biology and Biochemistry*, **26**(1), 29-39.
- Ryden J C. (1986). Gaseous losses from a grassland soil and the atmosphere. *Nature*, **292**, 235-237.
- Saggar S., Andrew R. M., Tate K. R., Hedley C. B., Rodda N. J., Townsend J. A. (2004a). Modelling nitrous oxide emissions from dairy-grazed pastures. *Nutrient Cycling in Agroecosystems*, **68**(3), 243-255.

- Saggar S., Bolan N. S., Bhandral R., Hedley C. B., Luo J. (2004b). A review of emissions of methane, ammonia, and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. *New Zealand Journal of Agricultural Research*, **47**(4), 513-544.
- Saggar S., Jha N., Deslippe J., Bolan N. S., Luo J., Giltrap D. L., Kim D. G., Zaman M., Tillman R. W. (2013). Denitrification and  $\text{N}_2\text{O}:\text{N}_2$  production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. *Science of The Total Environment*, **465**, 173-195.
- Samad M. S., Bakken L. R., Nadeem S., Clough T. J., de Klein C. A. M., Richards K. G., Lanigan G. J., Morales S. E. (2016). High-Resolution Denitrification Kinetics in Pasture Soils Link  $\text{N}_2\text{O}$  Emissions to pH, and Denitrification to C Mineralization. *PLoS ONE*, **11**(3).
- Sanchez-Martín L., Meijide A., Garcia-Torres L., Vallejo A. (2010). Combination of drip irrigation and organic fertilizer for mitigating emissions of nitrogen oxides in semiarid climate. *Agriculture, Ecosystems & Environment*, **137**(1–2), 99-107.
- Sänger A., Geisseler D., Ludwig B. (2011). Effects of moisture and temperature on greenhouse gas emissions and C and N leaching losses in soil treated with biogas slurry. *Biology and Fertility of Soils*, **47**(3), 249-259.
- Scheer C., Grace P. R., Rowlings D. W., Payero J. (2013). Soil  $\text{N}_2\text{O}$  and  $\text{CO}_2$  emissions from cotton in Australia under varying irrigation management. *Nutrient Cycling in Agroecosystems*, **95**(1), 43-56.
- Scheer C., Grace P. R., Rowlings D. W., Payero J. (2012). Nitrous oxide emissions from irrigated wheat in Australia: impact of irrigation management. *Plant and Soil*, **359**(1), 351-362.
- Scheer C., Wassmann R., Klenzler K., Lbragimov N., Eschanov R. (2008b). Nitrous oxide emissions from fertilized irrigated cotton (*Gossypium hirsutum* L.) in the Aral Sea Basin, Uzbekistan: Influence of nitrogen applications and irrigation practices. *Soil Biology and Biochemistry*, **40**, 290-301.
- Scholefield D., Hawkins J. M. B., Jackson S. M. (1997). Use of a flowing helium atmosphere incubation technique to measure the effects of denitrification controls applied to intact cores of a clay soil. *Soil Biology and Biochemistry*, **29**(9), 1337-1344.
- Secretariat of the Pacific Regional Environment Programme (SPREP). (2014). Climate Change Overview. Retrieved from <https://sprep.org/Climate-Change/climate-change-overview>
- Sexstone A. J., Parkin T. B., Tiedje J. M. (1988). Denitrification response to soil wetting in aggregated and unaggregated soil. *Soil Biology and Biochemistry*, **20**(5), 767-769.
- Sherlock R. R., Muller C., Hendriksen S. D., Barringer J. R. F., Cameron K. C. (1997). Methodology for assessing nitrous oxide and methane fluxes from agricultural soils. Lincoln University, New Zealand.: Lincoln Soil Quality Research Centre.

- Šimek M., Cooper J. E. (2002). The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. *European Journal of Soil Science*, **53**(3), 345-354.
- Simojoki A., Jaakkola A. (2000). Effect of nitrogen fertilization, cropping and irrigation on soil air composition and nitrous oxide emission in a loamy clay. *European Journal of Soil Science*, **51**(3), 413-424.
- Smith K. A., Arah J. R. M. (1990). Losses of nitrogen by denitrification and emissions of nitrogen oxides from soils (pp. 34 pp.).
- Smith K. A., Thomson P. E., Clayton H., McTaggart I. P., Conen F. (1998). Effects of temperature, water content and nitrogen fertilisation on emissions of nitrous oxide by soils. *Atmospheric Environment*, **32**(19), 3301-3309.
- Soil Survey Staff. (1998). Keys to Soil Taxonomy, eighth ed. Washington, DC: United States Department of Agriculture.
- Statistics New Zealand. (2012). Agricultural production statistics: June 2012 (final). Wellington: Statistics New Zealand.
- Throbäck I. N., Enwall K., Jarvis Å., Hallin S. (2004). Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE. *FEMS Microbiology Ecology*, **49**(3), 401-417.
- Treweek G. (2015). *The effect of winter grazing and a nitrification inhibitor on nitrous oxide emissions and denitrification in a stony soil*. (PhD), Lincoln University Lincoln, New Zealand.
- Treweek G., Di H. J., Cameron K. C., Podolyan A. (2016a). Simulated animal trampling of a free-draining stony soil stimulated denitrifier growth and increased nitrous oxide emissions. *Soil Use and Management*, **32**(3), 455-464.
- Treweek G., Di H. J., Cameron K. C., Podolyan A. (2016b). Effectiveness of the nitrification inhibitor dicyandiamide and biochar to reduce nitrous oxide emissions. *New Zealand Journal of Agricultural Research*, **59**(2), 165-173.
- Trost B., Prochnow A., Drastig K., Meyer-Aurich A., Ellmer F., Baumecker M. (2013). Irrigation, soil organic carbon and N<sub>2</sub>O emissions. A review. *Agronomy for Sustainable Development*, **33**(4), 733-749.
- Unger S., Máguas C., Pereira J. S., David T. S., Werner C. (2010). The influence of precipitation pulses on soil respiration – Assessing the “Birch effect” by stable carbon isotopes. *Soil Biology and Biochemistry*, **42**(10), 1800-1810.
- United Nations Framework Convention on Climate Change (UNFCCC). (2015). The Paris Agreement. Retrieved from [http://unfccc.int/paris\\_agreement/items/9485.php](http://unfccc.int/paris_agreement/items/9485.php)



- Wang G., Liang Y., Zhang Q., Jha S. K., Gao Y., Shen X., Sun J., Duan A. (2016). Mitigated CH<sub>4</sub> and N<sub>2</sub>O emissions and improved irrigation water use efficiency in winter wheat field with surface drip irrigation in the North China Plain. *Agricultural Water Management*, **163**, 403-407.
- Weier K. L., Doran J. W., Power J. F., Walters D. T. (1993). Denitrification and the Dinitrogen/Nitrous Oxide Ratio as Affected by Soil Water, Available Carbon, and Nitrate. *Soil Science Society of America Journal*, **57**(1), 66-72.
- Wrage N., Velthof G. L., van Beusichem M. L., Oenema O. (2001). Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biology and Biochemistry*, **33**(12–13), 1723-1732.
- Zheng X., Wang M., Wang Y., Shen R., Gou J., Li J., Jin J., Li L. (2000). Impacts of soil moisture on nitrous oxide emission from croplands: a case study on the rice-based agro-ecosystem in Southeast China. *Chemosphere - Global Change Sci.*, **2**(2), 207-224.